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RELATION OF ROOT DISTRIBUTION TO ORGANIC MATTER IN PRAIRIE SOIL[1]

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(WITH ELEVEN FIGURES)

Introduction

Although the root distribution of the chief grasses and forbs of tall-grass prairie has been known for 15 years (**18**), no studies on the relation of root distribution to the organic matter in the soil have been made. In fact, so far as the writers are aware, few studies of this type have been undertaken in America. SPRAGUE (**15**), working in New Jersey, has attempted to correlate root occupation of the several soil layers with their specific soil properties. The grasses, chiefly Kentucky bluegrass and Colonial bent grass, were grown on a formerly cultivated soil of the gray-brown forest soil group of the humid eastern states. Practically all of the roots were found in the upper 9 inches of soil, the abundance decreasing rapidly with depth. He found no correlation between root distribution and organic carbon content of the soil.

During the late fall of 1932, the writers selected a representative square meter of typical grassland in each of the two most important types or consociations of tall-grass prairie. The soil was removed to the depth of penetration of the grasses for the purpose of determining the relation of root distribution to the organic matter in prairie soil.

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The roots were separated from the soil and the organic matter in both roots and soil in the several soil levels was determined.

Stations

Little bluestem (*Andropogon scoparius*) and big bluestem (*A. furcatus*) are the most important two of the six grassland types occurring in the eastern one-third of Nebraska and the western one-third of Iowa, but also including large areas in Kansas, Missouri, Minnesota, and South Dakota (**19**). These two consociations constitute fully 80 per cent of the tall-grass prairie of this region. Because of their importance, the stations were selected in these types.

A tract of 120 acres of upland prairie dominated by little bluestem and known as the Belmont prairie lies one mile north of Lincoln. The vegetation has been thoroughly studied and continuous records of the environmental factors during the growing season have been obtained over a period of 15 years (**20**).

An area of about 40 acres of nearly pure big bluestem prairie is located on the floodplain of the Missouri River near Union, about 40 miles east of Lincoln, Nebraska. This prairie, owned by Mr. George Everett, was selected as representative of the *Andropogon furcatus* consociation and carefully studied (**7, 19**).

Vegetation

The Belmont prairie covers rolling hills. Little bluestem constitutes about 60 per cent of the vegetation; needle grass (*Stipa spartea*), June grass (*Koeleria cristata*), and prairie drop seed (*Sporobolus heterolepis*) are other common bunch grasses. Big bluestem and Indian grass (*Sorghastrum nutans*) are important sod-forming species as is also Kentucky bluegrass (*Poa pratensis*) (fig. 1). Scribner's panic grass (*Panicum scribnerianum*) and Wilcox's panic grass (*P. wilcoxianum*) are common interstitial species. The basal or ground cover is about 15 per cent, although the foliage cover, which varies greatly from year to year and with the progress of the season, is usually between 75 and 100 per cent (fig. 2). Although the abundant forbs often form extensive societies, the grasses are everywhere the dominant species. In some local areas forbs are sparingly represented.

FIGS. 1, 2.—Fig. 1 (above), view in the *Andropogon scoparius* type of grassland late in June. Grass is about 15 inches high and *Psoralea floribunda* about 30 inches. Fig. 2 (below), detail of basal cover in upland prairie. Bunches of little bluestem have been cut and reveal about 85 per cent soil surface.

The Everett prairie occurs on nearly level, well drained land lying between the Missouri River and its western bluff (fig. 3). Big bluestem alone constitutes 85 to 90 per cent of the vegetation. The chief accompanying grasses are Indian grass (*Sorghastrum nutans*), tall panic grass (*Panicum virgatum*), and relict slough grass (*Spartina michauxiana*). As a result of continued annual mowing, bluegrass has invaded but only in relatively small amounts. These sod-forming grasses, with a sprinkling of forbs, form a tall, dense foliage cover although the basal cover is only about 12 per cent (fig. 4).

Growth

Growth of vegetation on both upland and lowland prairie is normally renewed about the middle of April, although certain species are somewhat earlier. Perhaps only 2 per cent of the vegetation consists of annual plants. The abundant accumulations of reserve foods in the underground parts of the perennials permit rapid development with the occurrence of warm weather in spring. Little bluestem attains a height of 3 to 4 inches early in May and a general level of about 18 inches late in July. The flower stalks then elongate rapidly and reach a height of 2 to 2.5 feet. FLORY (**6**) has determined its average percentage of growth by weight during a period of 3 years as follows: 2 per cent in April, 28 in May, 36 in June, and 21 in July. By the end of July it had practically completed vegetative growth and had the greatest functional surface of leaves. The 12 per cent of growth in August and the 1 per cent in September consisted almost entirely of flower-stalk production.

The roots of the little bluestem reach a depth of about 4 feet. The extent of deterioration of the old root system each year and the rate of its replacement as well as the addition of more roots are as yet unsolved problems. It is known, however, that an entirely new root system may develop to the usual depth in a single summer if a block of sod is transplanted (**4**). Upland grasses and forbs in general grow more slowly than do those of lowlands and they are of smaller stature.

Growth in the low prairie is rapid. Big bluestem, the most important dominant, elongates at a maximum rate of nearly 2 cm. per day, and like the other tall grasses completes its vegetative growth

FIGS. 3, 4.—Fig. 3 (above), general view of Everett prairie late in June. Grass is *Andropogon furcatus* and coarse herb, *Silphium integrifolium*. Fig. 4 (below), basal cover of a practically pure stand of *A. furcatus* on low prairie near Union, Nebraska. Plants cut and photographed late in June.

late in July. Then the flower stalks begin to develop, often elongating 4 to 8 cm. per day, and the plant reaches a height of 6 to 10 feet. The roots extend to near the water table at 7 to 8 feet. Other tall grasses grow at a similar rate, slough grass always exceeding the bluestem in height. The taller forbs grow even more rapidly than the grasses and continuously overtop them. The yield of hay ranges from 0.75 to 1 ton per acre in the little bluestem type; that of the big bluestem prairie is 1.5 tons or more per acre. The vegetation cut from the square meter of upland soil before root excavation had a dry weight of 438 gm.; that from the lowland 1135 gm. Grasses constituted 92 per cent of the yield from the upland quadrat and 94 per cent of that from the low prairie.

Habitat factors

The mean annual precipitation at Lincoln is about 28 inches; that at Nebraska City, a few miles south of the Everett prairie, is 34 inches. Its distribution is of the Great Plains type, between 76 and 79 per cent occurring between April 1 and September 30.

Water content in the surface 6 inches of upland soil varies widely and rapidly, often 10 per cent or more during a single week. It was reduced to less than 5 per cent in excess of the hygroscopic coefficient one to four times annually during eleven of the twelve years that samples were regularly taken (**20**). Only twice during this period was the water content of this layer reduced to the hygroscopic coefficient. At greater depths to 4 feet there was always some water in excess of the hygroscopic coefficient, the available supply usually ranging between 5 and 20 per cent. The greater amounts of available soil moisture at the Everett prairie during a year of approximately average rainfall are shown in figure 5.

The average day humidity at Belmont, during a period of 12 years, varied between 50 and 80 per cent during years of greater rainfall but fell frequently to 40 to 50 per cent during drier years. In the Everett prairie it was 10 to 15 per cent higher.

Evaporation from white, cylindrical, porous cup atmometers varied greatly from year to year. It rarely fell below an average weekly loss of 10 cc. per day and was usually between 20 and 30 cc.; during periods of drought it sometimes reached 40 to 55 cc. The

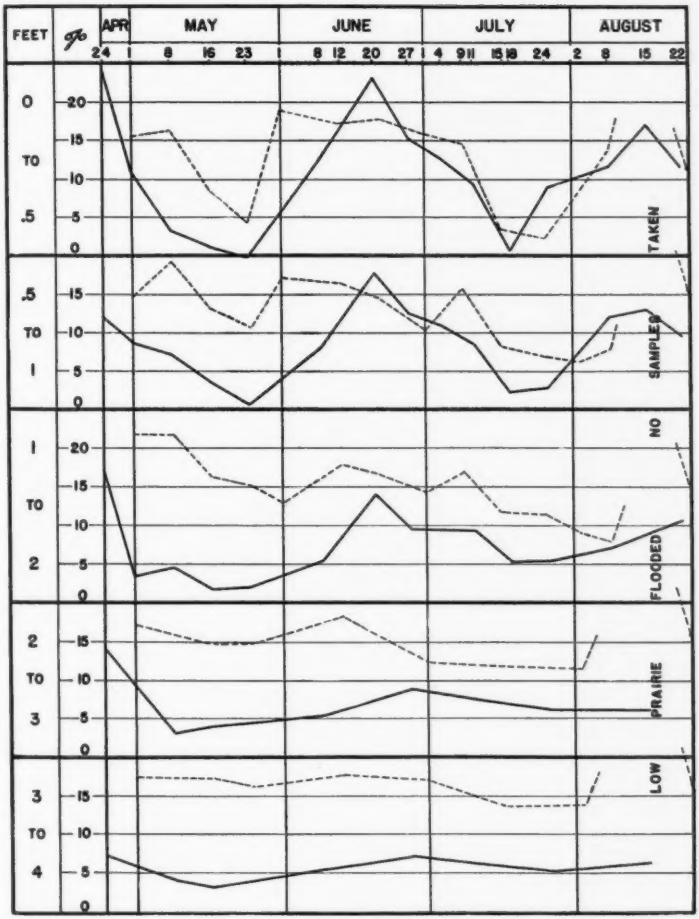


FIG. 5.—Percentage of water in excess of hygroscopic coefficient at Belmont station (solid line) and Everett prairie (broken line) at several depths during 1932.

average daily evaporation rate was considerably less in the lowland prairie at Union, as was also the wind movement.

Soils

The soil at the Belmont station is Lancaster loam, a mature upland soil of rolling topography derived from the Dakota sandstone formation. The samples were taken from a gentle north slope. The *A* horizon extends to a depth of about 16 inches; the *B* horizon lies between 18 and 30 inches; and the *C* horizon extends far beyond the range of penetration of the roots of the grasses. The surface soil is a very dark grayish brown loam about 12 inches in depth, thoroughly interlaced with grass roots, well supplied with organic matter, and has a highly developed fine granular structure. It contains noticeable amounts of medium and fine sand and is very friable. The soil at 12 to 16 inches forms a transition layer, grayish brown in color, friable, increasing in compactness, clay content, and size of structural aggregates, the latter grading up to 4 or 5 mm. in size. Below this is the horizon of clay accumulation which extends to a depth of 30 inches. It is friable, light grayish brown in color, and shows occasional rusty brown iron stains. Grass roots are numerous but small. The texture becomes much more sandy from the 30-inch depth to about 42 inches. There is little change in color except that rust stains and chalky spots are numerous. At the 42- to 44-inch level the sand is entirely rust-brown in color. Below this it is light grayish brown and is compact and cemented.

The soil of the Everett prairie is mapped as Wabash silt loam, a type frequently found in the bottomlands of eastern Nebraska. It is described as a dark grayish brown or dark brown to black, heavy, smooth silt loam, having an average depth of 24 inches, underlain by a more compact silt loam which is usually somewhat lighter in color (**8**). It is alluvial in origin; the topography is flat and the drainage was originally poor, but has been greatly improved by cleaning the drainage channels and constructing roads and ditches. Before the land was settled it was undoubtedly subject to frequent inundations by surface run-off from the higher land, and has been inundated, once to a depth of several feet, within the past 50 years. Mr. Everett has owned this prairie for more than 50 years and has seen

the vegetation gradually change, as the result of improved drainage conditions, from slough grass to big bluestem.

The profile examined was found to have a surface horizon, about 6 inches in depth, of dark grayish brown clay loam having a fine granular structure owing to the action of the fibrous grass roots with which this layer is heavily interspersed. Although this soil is mapped as silt loam, the high hygroscopic coefficients (18.2 and 19.7 for the first and second 6 inches respectively) indicate it to be much heavier in texture. It is a heavy clay loam. The 6- to 12-inch layer is less granular, lighter in color, and much lower in organic matter than the surface 6 inches. At the depth of 12 inches there is a layer one-eighth to one-fourth inch thick of very light grayish brown material, sandier than the deposits above and below.

At the depth of 12 to 24 inches there is a horizon whose nearly black color, highly developed fine granular structure, and comparatively great thickness indicate that it was formerly the surface soil for a long period of time. It has the morphological characteristics of a surface soil more fully developed than has the present surface foot. The physical and chemical analyses confirmed this field observation. Compared with the 6- to 12-inch horizon, the second foot was found to have smaller volume-weight and specific gravity and greater pore space, organic matter, and nitrogen content. The hygroscopic coefficient, 15.6, indicates it to be somewhat less heavy in texture than the surface layers. It is believed that many decades have elapsed since it was first covered with the present surface soil.

Between 24 and 28 inches there is a transition to a zone of light grayish brown soil which extends from 28 to 45 inches. A few gray and rust-brown mottlings are present, indicating imperfect drainage. Grass roots are in evidence, but structural units are only slightly developed. At 45 inches there is a rather abrupt transition to a dark gray material of massive structure containing somewhat more very fine sand and silt than the horizons above it. Within this horizon, which extends from 45 inches downward to the ground-water level, are strata of slightly sandier texture and lighter gray color. The darker color and slightly higher organic content indicate that the 45-inch horizon probably was once a surface soil for a long enough time to accumulate such a supply of organic matter. The slate-gray

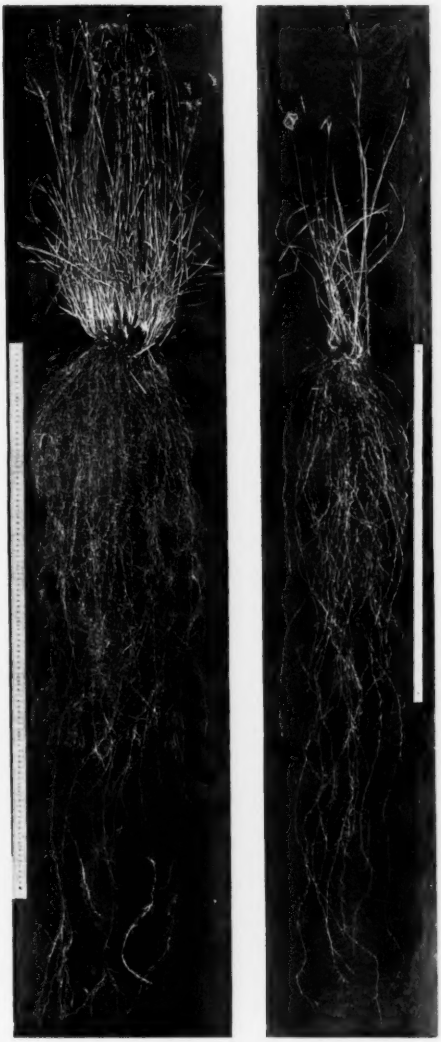
color indicates the deficiency of aeration and drainage, but the soil is now well penetrated by the roots of both grasses and non-grasses and the channels left by the decay of former generations of roots.

Root habits of plants excavated

The vegetation on the square meter selected for the excavation in the little bluestem prairie was about 87 per cent *Andropogon scoparius*. It was free from coarse-rooted forbs but supported small amounts of *Antennaria campestris* and *Aster multiflorus*. A few other grasses also occurred. These are so similar to little bluestem in root habit that the latter alone need be described. *Andropogon scoparius* developed a great mass of fine roots only 0.1 to 0.8 mm. in diameter. The roots were so abundant that they formed a dense sod. Many of them penetrated more or less vertically or obliquely downward, some reaching a depth of 4 feet. Others spread horizontally or obliquely in the surface soil. The surface foot was especially well occupied with dense masses of finely branched roots. They were also abundant in the second and third foot and even the fourth foot of soil was fairly well interspersed with fine roots, all of which were profusely branched (fig. 6).

The square meter selected for excavation on the big bluestem prairie contained, in addition to the dominant, a small amount of *Sorghastrum nutans* and a sprinkling of *Equisetum laevigatum*. The very abundant roots of *Andropogon furcatus* grew both vertically and obliquely downward, thoroughly occupying the soil and forming a dense sod. The larger roots varied from 0.5 to 3 mm. in diameter and a few reached a depth of 7 feet. The roots tapered so gradually that at 4 feet they were nearly as large as at the surface. All branched profusely even to near their tips (fig. 7). The roots of *Sorghastrum nutans* were similar in habit but did not penetrate so deeply.

The underground parts of *Equisetum laevigatum* were of special interest. They bulked large in amount, and have heretofore been undescribed. The tops originated from a much branched system of underground stems, which in the upper 2.5 feet of soil were mostly vertical or only slightly oblique. But from 2.5 to about 5.5 feet the stems grew horizontally or obliquely for long distances, giving rise



FIGS. 6, 7.—Fig. 6 (left), roots and tops of mature *Andropogon scoparius* from high prairie; fig. 7 (right), roots and tops of mature *A. furcatus* from low prairie. Scale in both figures is a meter stick.

to many branches which extended more or less vertically upward or downward. Some plants, however, were found that grew vertically downward to 7 feet with little or no branching. Usually the horizontal branching habit and an irregular downward course were characteristic features. Below 5.5 feet there were few horizontal branches, and even the vertically penetrating ones were much less abundant. The stemlike underground parts were of a steel blue or black color, distinctly jointed, and except for threadlike roots were like the parts above ground. The roots arose from the nodes or near them, often in groups of two or three but sometimes singly. A few were branched. None was found that exceeded 2.5 inches in length. In the deeper soil (usually below 5.5 feet) a type of branch arose from the horizontal stems that was apparently free from nodes, although the internal pattern, as observed with a hand lens, was similar to that of the stems. Moreover the short laterals arose irregularly all along the branch, sometimes at the rate of twelve per inch. While the underground stems were 6 to 7 mm. in diameter these roots were often only 4 to 5 mm. thick. Some were found that were thickly covered with a brown tomentum about 5 mm. long resembling root hairs. Numerous roots penetrated to a depth of 7.5 feet.

All roots were separated from the soil by a process of repeated washing, after the blocks of soil had been transported to the greenhouse. It was estimated that less than 1 per cent of the roots was lost, a negligible amount considering the variation in root distribution in the prairie.

Methods of analysis

Soil samples for the volume-weight determinations were taken by means of special sampling equipment in use at the Nebraska Experiment Station. This equipment consists of a steel tube 4 inches in inside diameter and 2 feet long, fitted with a lining of easily removable brass cylinders, each 6 inches long. On the lower end of the steel tube is brazed a cutting ring whose inside diameter corresponds exactly with that of the brass linings. The tube is forced into the soil by means of a jack. Large augers are set in the ground about 30 inches apart, with a wooden span extending between them to serve as an anchor for the jack. A complete description of the apparatus

will be published soon (**10**). The field moisture contents and the volume-weights in table I were determined from 6-inch sections. The figures for the first and second 6-inch samples are the results of single determinations; those for all other depths are the average of the two 6-inch sections of each foot section. In each case the variation between the volume-weights of the two 6-inch sections was not more than a few hundredths.

The samples for the determination of organic matter and nitrogen and those for the determination of volume-weight were taken only a foot or two distant from the excavation made in securing the soil containing the roots. The samples for organic matter and nitrogen amounted in each case to several kilograms. They were dried and screened through a sieve of 4 meshes per inch, all roots and rhizomes being cut into pieces and mixed with the soil without loss. After thorough mixing of the large bulk sample, a 1 pint subsample was taken. The pint sample thus obtained was passed through a 2 mm. sieve, all roots being ground and retained as before. No gravel larger than 2 mm. in diameter was found in any of the samples, except an insignificant amount (less than 0.2 per cent) in the 2- to 4-foot depths of the Lancaster profile; these larger particles were discarded. The pint sample was again subsampled, about 100 gm. being taken and ground to pass through a 1 mm. sieve. This was used for the moisture equivalent and hygroscopic moisture determinations. Another 100 gm. portion was ground to pass through a 0.25 mm. sieve for the determinations of organic matter, nitrogen, and specific gravity.

The root materials were ground in a Wiley mill with a 2 mm. sieve. The organic matter of the roots was determined by ignition in an electric furnace. Nitrogen in the roots and soils was determined by the usual Gunning method (**3**). Organic matter in the soils was determined by a modification of the hydrogen peroxide method of ROBINSON (**9**). Duplicate determinations agreed within a few hundredths of 1 per cent. RUSSEL and ENGLE (**12**) have shown that the method is reliable and yields results agreeing closely with those obtained by multiplying organic carbon content by the conventional factor 1.724.

The hygroscopic coefficient was determined by the method of

ALWAY, KLINE, and MCDOLE (1). The moisture equivalent was determined as described by RUSSEL and BURR (11). The specific gravity determinations were made by means of the pycnometer. The samples of both soils and roots for moisture determination were dried at 110° C.; the organic matter, nitrogen, and moisture percentages were calculated on the basis of oven-dry sample weights.

Results

The moisture and volume relations of the soils are shown in table I. Mechanical analyses were not made, since the soil texture is clearly indicated by the hygroscopic coefficients.

TABLE I

MOISTURE AND VOLUME RELATIONS IN THE SOILS

DEPTH (FEET OR INCHES)	HYGROSCOPIC COEFFICIENT (%)	FIELD MOISTURE CONTENT (F.M.C.) (%)	MOISTURE EQUIVALENT (%)	VOLUME-WEIGHT (GM./CC.)	SPECIFIC GRAVITY	PORE SPACE (%)	SPACE OCCUPIED BY WATER AT F.M.C. (%)	AIR SPACE AT F.M.C. (%)
	Lancaster loam at Belmont prairie							
0–6″	11.0	30.4	35.4	1.12	2.59	56.8	34.1	22.7
6–12″	12.8	21.9	41.0	1.31	2.62	50.0	28.7	21.3
1–2′	12.6	14.9	37.8	1.41	2.63	46.4	21.0	25.4
2–3′	9.6	15.7	28.7	1.55	2.70	42.6	24.3	18.3
3–4′	5.6	13.5	14.8	1.64	2.68	38.8	22.1	16.7
	Wabash clay loam at Everett prairie							
0–6″	18.2	20.7	41.9	1.05	2.60	59.6	21.7	37.9
6–12″	19.7	24.2	43.4	1.16	2.68	56.7	28.1	28.6
1–2′	15.6	23.2	36.1	1.10	2.61	57.9	25.5	32.4
2–3′	14.6	25.8	34.1	1.19	2.66	55.3	30.7	24.6
3–4′	15.8	29.2	36.1	1.20	2.66	54.9	35.0	19.9
4–5′	15.6	32.6	36.2	1.27	2.65	52.1	41.4	10.7
5–6′	14.2	31.4	35.5	1.24	2.66	53.4	38.9	14.5
6–7′	12.8	30.8	34.0	1.33	2.69	50.6	41.0	9.6

TEXTURE.—The figure 11.0 for the surface 6 inches of the Lancaster loam indicates a rather heavy loam texture; the greater hygroscopic coefficients of the 6- to 24-inch layer and the decreasing

figures below this depth agree well with the field observation of a clay horizon in the second foot and an increasing sandiness below this horizon.

In the Wabash clay loam the variations in hygroscopic coefficients may be taken to indicate corresponding variations in texture, the second 6 inches being the most clayey and the seventh foot the least clayey in the profile. Since this is an alluvial soil, no consistent variations can be found in the hygroscopic coefficients as the result of weathering processes in the profile, the texture of the deposited material having overshadowed any possible effects of weathering.

MOISTURE EQUIVALENT.—The moisture equivalents of the Lancaster soil samples are approximately three times the hygroscopic coefficients; those of the Wabash soils are approximately 2.5 times the hygroscopic coefficients. The moisture equivalent gives an approximate indication of the field moisture carrying capacity of the soil but exceeds it appreciably in soils of heavy texture. For example, the field moisture contents of the surface 6 inches of Lancaster loam, and of the lower 4 feet of the Wabash clay loam, were probably close to the field carrying capacity at the time of sampling, but they were appreciably lower than the moisture equivalents. Both soils had been dried considerably by the grass roots, as is indicated by their field moisture contents at the time of sampling, which are shown in table I.

VOLUME-WEIGHT.—The volume-weight of a soil is the weight of dry soil per unit volume. It is expressed in table I as grams per cubic centimeter. The volume-weight of the surface 6 inches of the Lancaster loam (1.12) may be considered a typical figure for virgin prairie soils of medium texture. It is low in comparison with that of the deeper horizons because of the high degree of granulation and the presence of a large amount of root material in the surface soil. The increasing volume-weight with increasing depth is correlated with decreasing granulation and also with increasing sandiness of texture. The figures 1.55 and 1.64 for the third and fourth foot sections indicate a high degree of compactness.

The volume-weight of the surface section of the Wabash soil is exceptionally low, owing to the highly granular structure of this horizon and the presence of a large volume of roots and rhizomes. The

volume-weight of the second foot is noticeably lower than that of the second 6 inches; it corresponds to that of a surface soil. This fact is in harmony with the field observation that the second-foot section is a buried surface soil. Below this level the volume-weight increased consistently with greater depth and less intensive root penetration and weathering.

SPECIFIC GRAVITY.—The specific gravity of the soil is influenced to some extent by the organic content. This fact is well illustrated by the figures given in table I. In the Lancaster loam the specific gravity increased with depth as the organic matter decreased. In the Wabash clay loam the specific gravity of the second-foot section was lower than that of the horizons above or below, corresponding with the higher organic content of this section. Below the 3-foot level the variations in specific gravity in this profile were of no significance.

PORE SPACE.—The pore space of the soil was calculated from the formula

$$P = 100 - \left(\frac{100\ V}{S}\right),$$

in which P = per cent pore space, V = volume-weight, and S = specific gravity. The pore space decreases with depth as a general rule, being mainly an inverse function of the volume-weight. This relation is more obvious in the case of the Lancaster loam because of the sandier texture of the deeper horizons. Sandy soils as a rule have greater volume-weight and less pore space than heavy soils.

The percentage of the total soil volume occupied by water at any water content is readily calculated by multiplying the percentage of water on the dry weight basis by the volume-weight. This method has been used to calculate the percentage of the total volume of soil occupied by water at the field moisture content at the time of sampling. The difference between the total pore space and the space occupied by water is the percentage of air space in the soil at the water content in question. In figures 8 and 9 the air space is based upon the average water content of the soil during the season of 1932.

The data on air space at field moisture content in the Lancaster

loam indicate a well aerated profile throughout. The decrease in air space at the lower depths is due to the sandier texture and consequent smaller total pore space, while the decrease in air space in the deeper horizons of the Wabash soil is the result of higher water content. Even in the seventh foot, however, which was just above the ground-water level, the pore space of the soil was not entirely filled

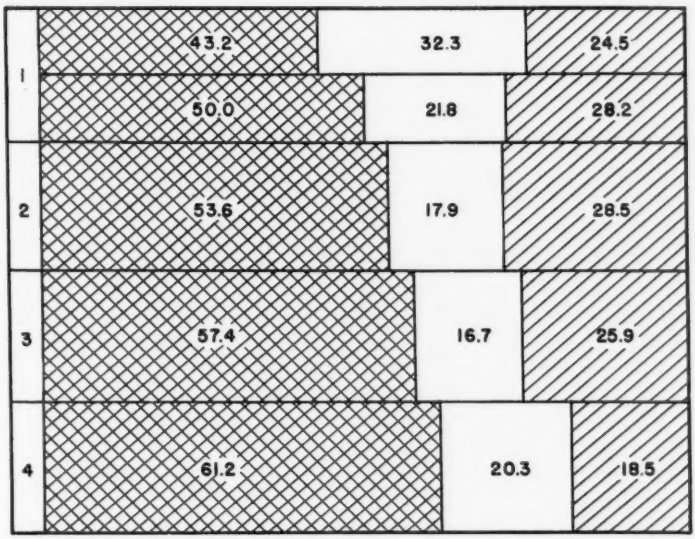


FIG. 8.—Diagram showing percentage of solid matter (cross hatch) and total pore space in first 4 feet of Lancaster loam soil covered with *Andropogon scoparius*. Portion of pore space occupied by water (single hatch) is based upon average water content during 1932; unhatched part is air space.

with water; about 10 per cent of the soil volume, or one-fifth of the total pore space, was occupied by air.

ORGANIC MATTER AND NITROGEN OF SOILS.—In table II are presented the soil organic matter and nitrogen relations in the two profiles. The nitrogen in the soil organic matter (fifth column) is the reciprocal of the ratio of organic matter to nitrogen (fourth column) and is added for the convenience of those who are accustomed to think of the relation between nitrogen and organic matter on a percentage basis rather than as a ratio. The carbon-nitrogen

ratio (sixth column) has been calculated from the organic matter-nitrogen ratio by dividing by the conventional factor 1.724. It is

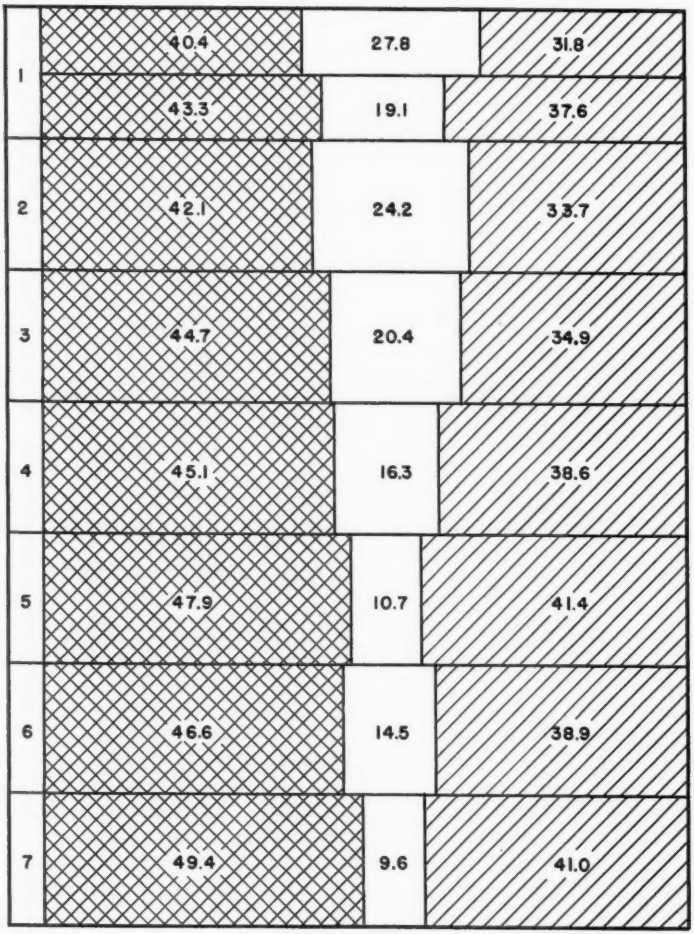


FIG. 9.—Diagram showing percentage of solid matter, air space, and water in the first 7 feet of Wabash clay loam soil covered with *Andropogon furcatus*. Legend and conditions as in figure 8.

presented for the purpose of comparison with other carbon-nitrogen ratios in the literature.

The weight of soil per square meter (seventh column) has been calculated from the volume-weight. The first two figures for each soil are for 6-inch sections, while the other figures correspond to 1-foot sections. Since the volume of one square meter to the depth of 6 inches is 152,400 cc., the weight of soil in grams per square meter for the 6-inch sections is obtained by multiplying this figure by the volume-weight; for the foot sections, weight per square meter equals

TABLE II

ORGANIC MATTER AND NITROGEN RELATIONS IN THE SOILS

DEPTH (FEET OR INCHES)	ORGANIC MATTER (%)	NITROGEN (%)	RATIO OM/N	NITROGEN IN SOIL OM (%)	RATIO C/N	WT. OF SOIL PER SQ.M. IN KG.	ORGANIC MATTER (GM./SQ. M.)	NITROGEN (GM./SQ. M.)
	Lancaster loam at Belmont prairie							
0–6″	4.50	0.224	20.0	5.0	11.6	171	7670	382
6–12″	3.24	.167	19.4	5.2	11.3	200	6480	334
1–2′	1.59	.093	17.1	5.8	9.9	430	6840	400
2–3′	0.43	.043	10.0	10.0	5.8	472	2030	203
3–4′	0.20	.024	8.3	12.0	4.8	500	1000	120
	Wabash clay loam at Everett prairie							
0–6″	4.92	.262	18.8	5.3	10.9	160	7880	420
6–12″	2.24	.138	16.2	6.2	9.4	177	3960	244
1–2′	2.77	.167	16.6	6.0	9.6	335	9280	560
2–3′	1.75	.114	15.3	6.5	8.9	363	6340	414
3–4′	1.24	.088	14.1	7.1	8.2	366	4540	322
4–5′	1.44	.078	18.5	5.4	10.7	387	5580	302
5–6′	1.22	.074	16.5	6.1	9.6	378	4600	280
6–7′	1.02	0.061	16.7	6.0	9.7	405	4140	248

volume-weight × 152,400 × 2. The weights given in the table are expressed in kilograms. These figures were used in converting the percentage of organic matter and nitrogen in the soil to grams per square meter in order to obtain the weight of soil organic matter and nitrogen in the volume of soil from which the roots were taken, as shown in the last two columns of table II.

The organic matter and nitrogen data for the Lancaster loam show the profile characteristics usually found in normal grassland soils. According to the extensive work of ALWAY and MCDOLE (2) and of

RUSSEL and MCRUER (13) in grassland soils, the nitrogen and organic matter decrease regularly with depth, the bulk of the organic matter being confined to the surface foot. In the first and second 6-inch sections, the organic matter and nitrogen contents are in agreement with those found by these investigators for soils of this texture in this part of the state; in the lower sections the organic content decreases more rapidly as a result of their sandy texture. The organic matter-nitrogen ratios throughout the profile are typical of the upland soils of this region.

The percentage of organic matter and nitrogen in the second foot of the Wabash silt loam is considerably higher than that in the 6-inch section just above it, correlating perfectly with the field observation that this layer shows the characteristics of a well developed surface soil. In virgin prairie soils, a higher nitrogen content is never found in a lower layer than in layers above, except in cases of buried soil profiles or where silting by wind or water has occurred comparatively recently (13). The ratio of organic matter to nitrogen shows a tendency toward that of the surface soil, for although the ratio 16.6 is perhaps not significantly higher than the ratio 16.2 in the second 6 inches, yet if the profile showed the characteristics of a mature soil, the ratio in the second foot should be significantly lower than 16.2. This is illustrated by the organic matter-nitrogen ratios of the Lancaster loam.

The relatively high values for the organic matter-nitrogen ratios of the lower 3 feet of the profile are believed to indicate that the 4-foot level also was at one time a surface soil. The slightly higher organic content of the fifth foot in comparison with the fourth foot is in agreement with this conclusion. In the field a rather abrupt change in the appearance and properties of the soil was observed at approximately the 4-foot level.

It seems remarkable that the soil organic matter should retain its characteristics after being buried and subjected to decomposition and weathering for a period that was undoubtedly several hundred years in duration, but the data in table II indicate that such is the case.

DRY WEIGHT, ORGANIC MATTER, AND NITROGEN OF ROOTS.—The dry weight, organic matter, and nitrogen of the roots recovered from

each section of the Lancaster loam are shown in table III. Nearly 60 per cent of the total root system was found in the upper 6 inches of soil. This, however, includes the rhizomes.

The high organic matter of the roots and short rhizomes of the little bluestem in the surface foot of soil is probably due to the presence of stored food reserves. The lower figures in the second and third foot may be due to decortication of the older roots in these sections, while the increase in the fourth foot may result from this portion of the root system containing the younger and smaller root-ends which often have a relatively larger proportion of living tissues.

TABLE III

DRY WEIGHT, ORGANIC MATTER, AND NITROGEN IN ROOTS FROM ONE SQUARE METER OF LANCASTER LOAM

DEPTH (FEET OR INCHES)	DRY WEIGHT (GM.)	ORGANIC MATTER (%)	NITROGEN (%)	ORGANIC MATTER (GM.)	NITROGEN (GM.)
0–6″	741.0*	90.8	0.828	657	6.13
6–12″	198.0	91.7	0.699	182	1.39
1–2′	193.0	83.1	0.656	160	1.26
2–3′	85.5	86.6	0.730	74	0.62
3–4′	12.0	91.4	0.800	11	0.10

* This includes also the rhizomes of the grasses all of which occurred in the surface 6 inches of soil.

In the Wabash profile it was possible to separate the grass roots from the non-grasses and in the surface layer to separate the grass roots from the rhizomes. The dry weight, organic matter, and nitrogen in these roots and rhizomes are shown in table IV. In the fifth foot and deeper, the quantity of grass roots alone was too small for analysis, hence the grasses and non-grasses were mixed. The organic matter in the non-grasses above the fifth foot varied from 78 to 88 per cent, with no consistent relation to depth. The high organic matter and nitrogen content of the rhizomes, 94 and 0.73 per cent respectively, may be attributed to their store of food reserves. The organic matter of the grass roots in the surface 6 inches, 74 per cent, was found to be considerably lower than in the deeper horizons, where the figure varied from 81 to 91 per cent. This may be due to the high degree of decortication of the older roots in the surface layer and to the leaching of soluble organic matter from the dead and

partly decayed roots. Unfortunately the roots and rhizomes of the little bluestem in the Lancaster soil were not separated, and the

TABLE IV

DRY WEIGHT, ORGANIC MATTER, AND NITROGEN IN ROOTS FROM ONE SQUARE METER OF WABASH CLAY LOAM

DEPTH (FEET OR INCHES)	KIND OF ROOTS, ETC.	DRY WEIGHT (GM.)	ORGANIC MATTER (%)	NITROGEN (%)	ORGANIC MATTER (GM.)	NITROGEN (GM.)
0–6″	Non-grasses*	23.2	84.3	0.594	19.6	0.138
	Grasses	726	74.3	.515	539	3.74
	Rhizomes of grasses	292	94.0	.728	274	2.12
	Total	1041	.....	.....	833	6.00
6–12″	Non-grasses	12.4	86.0	.372	10.6	.046
	Grasses	168	84.6	.500	142	.838
	Total	180	.....	.....	153	.884
1–2′	Non-grasses	34.0	78.3	.340	26.6	0.116
	Grasses	179	81.0	.532	145	.954
	Total	213	.....	.....	172	1.07
2–3′	Non-grasses	43.6	86.3	.301	37.6	.132
	Grasses	80.2	87.8	.514	70.8	.412
	Total	124	.....	.....	108	0.544
3–4′	Non-grasses	22.0	88.0	.305	19.4	.066
	Grasses	42.2	90.8	.456	38.2	.194
	Total	64.2	.....	.....	57.6	.260
4–5′	Non-grasses	42.6	.....	.....	.....	.....
	Grasses	14.0	.....	.....	.....	.....
	Total	56.6	87.4	0.332	49.4	.188
5–6′	Non-grasses	11.4	.....	.....	.....	.....
	Grasses	2.6	.....	.....	.....	.....
	Total	14.0	90.2	.342	12.6	0.048
6–7′	Non-grasses	11.4	.....	.....	.....	.....
	Grasses	0.6	.....	.....	.....	.....
	Total	12.0	78.1	0.409	9.4	0.049

* Non-grasses are very largely *Equisetum laevigatum*.

separate composition of each of these structures alone is not known.

The data in table V are taken from the preceding tables. They

permit several conclusions to be drawn concerning the relationships between the depth in the soil profile, the quantity of organic matter present, the quantity of roots, and the type of decomposition of the dead root material. It should be borne in mind that the "soil organic matter" of the tables includes that of the root material. This is the case because in the routine preparation of soil samples for analysis it is not practicable to remove all of the root material from the soil,

TABLE V

ORGANIC MATTER AND NITROGEN RELATIONS BETWEEN SOILS AND ROOTS

Depth (feet or inches)	Organic matter (gm.)		Ratio soil OM / root OM	Nitrogen (gm.)		Ratio soil N / root N
	in soils	in roots		in soils	in roots	
	Lancaster loam at Belmont prairie					
0–6″	7670	657	11.7	382	6.13	62
6–12″	6480	182	35.7	334	1.39	241
1–2′	6840	160	42.7	400	1.26	316
2–3′	2030	74.0	27.4	203	0.62	325
3–4′	1000	11.0	90.9	120	0.10	1250
	Wabash clay loam at Everett prairie					
0–6″	7880	833	9.46	420	6.00	70
6–12″	3960	152	26.0	244	0.88	276
1–2′	9280	172	54.0	560	1.07	523
2–3′	6340	108	58.5	414	0.544	765
3–4′	4540	57.6	78.9	322	.260	1210
4–5′	5580	49.4	113	302	.188	1590
5–6′	4600	12.6	365	280	.048	5880
6–7′	4140	9.4	440	248	.049	5020

nor is it possible to remove a part of this material without introducing the factor of personal judgment as to how much of the coarse roots should be sifted out and how much should be retained. The only simple method of avoiding the personal factor is to retain all of the root material, and this is the usual procedure in obtaining and preparing soil samples for analysis.

In the surface soil, according to table V, approximately one-tenth of the "soil organic matter" consists of plant roots and rhizomes, so that whether or not they are included as a part of the soil sample is a

matter of some importance. In the deeper layers the roots are of relatively less importance as a part of the organic matter of the soil. Figure 10 shows graphically the relations between the depth in the soil and the ratio of soil organic matter to the organic matter of the roots throughout each of the two profiles, and illustrates the fact that with increasing depth the mass of the roots becomes decreasingly important in proportion to that of the soil organic matter. The ratio of soil organic matter to the organic matter of the roots is roughly a linear function of the depth in the Lancaster profile. This is also true in the Wabash profile to the depth of 5 feet, but in the sixth and seventh foot the quantity of soil organic matter is far out of proportion to the quantity of roots. This is probably due to the excessive water content and deficient aeration which tend to inhibit the complete decomposition of organic matter and preserve it in the soil in the partly decomposed or humified state. The same statements apply to the relation between the depth in the profile and the ratio of soil nitrogen to root nitrogen which is not shown graphically because of its similarity to the organic matter relation.

The point corresponding to the 3-foot depth in the Lancaster soil deviates widely from the other points in this profile with respect to both organic matter and nitrogen ratios. This is believed to be due to the fact that the third foot is a sandy horizon, very permeable to grass roots, and capable of holding sufficient water and air for the rapid decomposition of dead roots. These conditions might be expected to result in the presence of a relatively large mass of living roots and a small amount of dead residues.

The quantity of roots found at any level in the soil depends to some extent on the fertility and physical properties of the soil, but is principally dependent upon the nature of the plant, unless the soil departs widely in parts of the profile from the conditions of texture, structure, fertility, and moisture usually found in the grassland soils of this region. In the case of the Wabash soil, it is evident that the organic matter of the soil has had no great influence on root development, for the 6- to 12-inch level contained 2.24 per cent of organic matter and 0.10 per cent of root material, while the 1- to 2-foot section contained 2.77 per cent of organic matter and only 0.06 per cent of root material. The lower layer of soil, relatively one-fifth

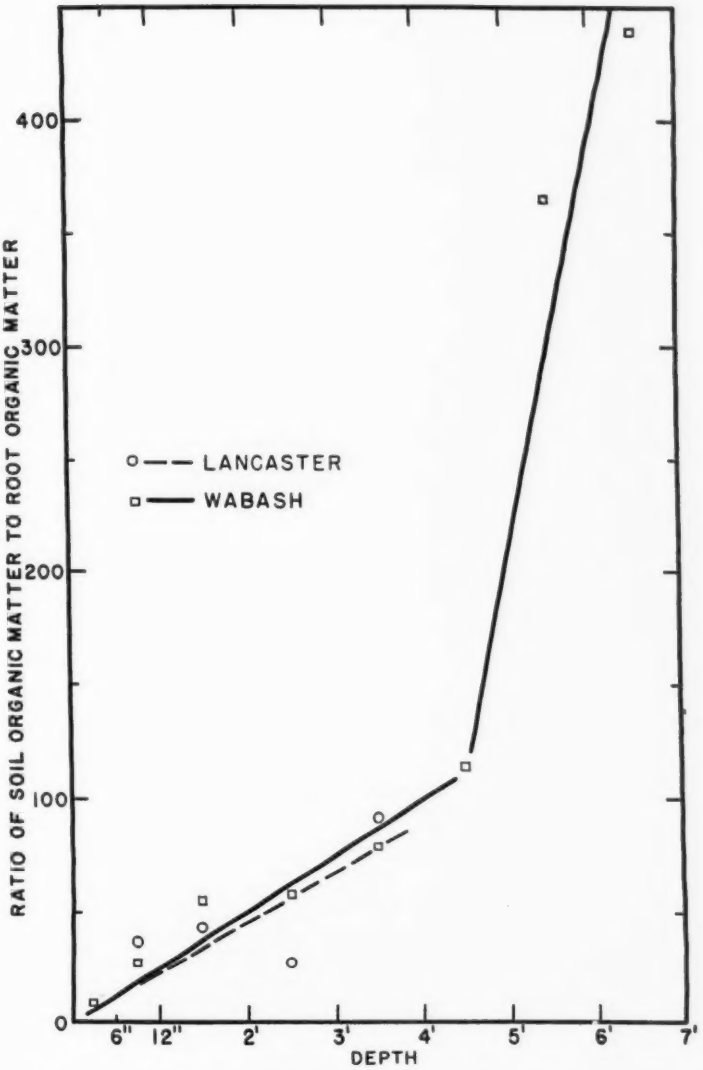


FIG. 1c.—Relation between depth in profile and ratio of soil organic matter to root organic matter.

richer in total organic matter, was relatively two-fifths poorer in root material.

The relation between the amount of organic matter in the soil and that in the roots is shown graphically in figure 11. The data for the organic matter of both soil and roots in the first and second 6-inch sections have been multiplied by two in order to place all the data on

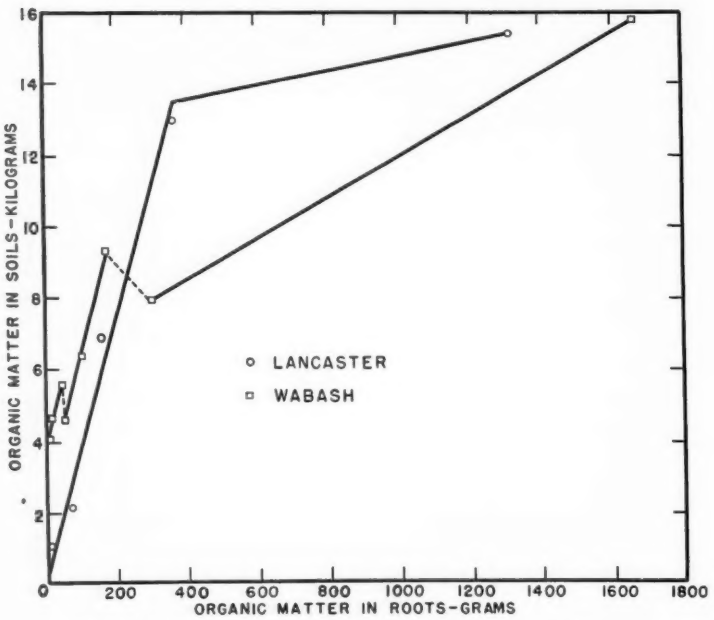


FIG. 11.—Relation of organic matter in roots to organic matter in soils.

the foot-section basis. The graph for the Lancaster soil is approximately a straight-line function except in the surface 6 inches, where the large mass of roots far exceeds the proportionate amount of soil organic matter. In the Wabash soil, however, there are breaks in the curve, corresponding to the positions of the buried surface soils in the profile. Between these points of discontinuity the slope of the curve is approximately the same as for the Lancaster soil. Although it is not advisable to make a positive statement based on the study

of but two profiles, these results suggest a possible constant relation, except in the surface layer of soil, between root development and soil organic matter.

Discussion

The nitrogen content of the soil organic matter is dependent on the climatic and soil conditions, and the character and amount of plant growth. Under virgin conditions the plant growth ultimately reaches an equilibrium which is the result of the interaction of the climatic conditions, the soil conditions, and the plant growth itself. The nitrogen content of the soil organic matter, which may also be expressed in the form of the carbon-nitrogen ratio or the organic matter-nitrogen ratio, is thus the result of an equilibrium between the accumulation of plant residues and their decomposition by molds and bacteria in the soil.

WAKSMAN (16) has explained the relatively constant carbon-nitrogen ratio of the soil organic matter and its significance as follows:

"It could not be otherwise; were the carbon content too large in relation to the nitrogen, the soil would not be in a condition to support plant growth, as long as this excess lasted; the microorganisms using the carbon as a source of energy would assimilate every trace of available nitrogen that would otherwise be made available for the growth of higher plants. Were the nitrogen content too large, ammonia would be rapidly liberated and then transformed to nitrates and either leached out or assimilated by higher plants."

WAKSMAN has estimated the carbon-nitrogen ratio of fungus mycelia to be about 9 or 10, and that of bacteria about 4 or 5. Undecomposed plant material has a ratio varying from 20 in legumes to 80 or more in wheat straw. The carbon-nitrogen ratio of the soil may thus be considered an index of the relative amounts of undecomposed plant residues, fungi, and bacteria present. SIEVERS and HOLTZ (14) have called attention to the fact that virgin soils have a wide ratio and that under cultivation the ratio tends to become narrower, as illustrated by the following table:

Virgin soil.....	13.1	12.5	11.3	14.0
Cropped 40 years.....	9.4	11.3	11.0	12.0

The carbon-nitrogen ratio in cultivated or fallowed soils, according to these investigators, tends to approach 9 as a minimum value. This is due mainly to the smaller supply of decomposable material returned to the soil each year, and probably to some extent to the more favorable conditions for decay in the cultivated soil.

ALWAY and MCDOLE (**2**) have reported the carbon-nitrogen ratios in successive foot-sections of virgin soil at Lincoln to be 12.0, 10.2, 9.5, 5.9, 6.0, and 5.4. In table II are presented the organic matter-nitrogen ratios and the corresponding carbon-nitrogen ratios of the several horizons of the Lancaster loam. The ratios decrease rapidly with increasing depth, and in this respect may be considered typical of normal upland prairie soil profiles. The narrower ratios at greater depths may be explained by the presence of relatively small quantities of root residues in proportion to relatively large quantities of the decomposition products of fungal and bacterial activity. BROWN and BENTON (**5**) have made a comprehensive study of the distribution of microorganisms in the prairie soils of Iowa. They have shown that the bacteria and actinomyces as a rule far outnumber the molds throughout the profile. The predominance of the bacteria and actinomyces tends to keep the carbon-nitrogen ratio low in those parts of the soil where the organic residues that form the food supply of the organisms are small in amount.

The organic matter-nitrogen ratios throughout the Wabash profile, shown in table II, are essentially those of surface soil. They appear to indicate that where the surface soil has accumulated organic matter and then been buried under fresh sediments, the organic matter so buried retains the characteristics of surface material for a long time. The explanation for such behavior is difficult to find. It may be tentatively suggested, without definite experimental proof applied to the conditions existing in these soils, that the surface type of organic matter contains a large proportion of lignins and lignin-like materials, non-nitrogenous substances which are resistant to decay. According to WAKSMAN and IYER (**17**), the lignins will combine with proteins, forming complexes that are highly resistant to decomposition, and which may be expected to persist in the soil unchanged for a long time. Decomposition would be especially slow in soils nearly saturated with water, as in the lower layers of the

Wabash profile. Additional evidence to support this theory is found in the fact that these layers contain relatively large amounts of organic matter in comparison with the corresponding sections of upland soils. The Wabash profile contains 1.24, 1.44, and 1.22 per cent of organic matter in the fourth, fifth, and sixth foot sections respectively, while the corresponding sections of loess soil at Lincoln were reported by ALWAY and MCDOLE (2) to contain 0.60, 0.43, and 0.40 per cent of organic matter.

Summary

1. A typical square meter of vegetation was selected in the upland, *Andropogon scoparius* prairie on Lancaster loam soil, near Lincoln, Nebraska, and another in the lowland, *A. furcatus* prairie on the Wabash clay loam soil of the floodplain of the Missouri River.

2. The surface soil was removed in 6-inch layers and the deeper soil in foot sections to the depth of root penetration; the roots and rhizomes were carefully removed by washing, and their dry weights, nitrogen contents, and organic contents determined.

3. Water content of soil and atmospheric factors affecting plant development were measured and rate of growth of the vegetation was determined. Root habits of the plants excavated were noted.

4. The soils varied so uniformly in texture, structure, and fertility with depth, that (except in buried surface layers) root distribution was of the usual type found for these species over a wide range of prairie soils.

5. In the Lancaster loam 60 per cent of the underground parts of the dominant grass was found in the surface 6 inches; the remainder of the root system was distributed to a depth of 4 feet. In the Wabash clay loam 68 per cent of the underground parts (60 per cent excluding rhizomes) was found in the surface 6 inches; the remainder of the root system extended to the depth of 7 feet and nearly to the water table.

6. The hygroscopic coefficient and moisture equivalent of each section of the soil profile were determined as indexes of the texture. The volume-weight of the soil in the undisturbed field condition was determined. From the volume-weight, specific gravity, and field moisture content at the time of sampling, it was possible to calculate

the pore space, space occupied by water, and air space in each section of the soil.

7. Volume-weight in the Lancaster loam increased gradually from 1.12 in the surface 6 inches, where an abundance of roots, rhizomes, and dead organic matter filled the soil, to 1.64 in the fourth foot, where both living roots and dead organic matter were relatively sparse.

8. Volume-weight in the Wabash clay loam increased gradually (except in buried surface soils) from 1.05 in the first 6 inches to 1.33 in the seventh foot. This was accompanied by a gradual decrease in both living root materials and dead organic matter.

9. Pore space in the surface 6 inches of Lancaster loam constituted 57 per cent of the volume of the soil. On an average 25 per cent was occupied by water and 32 per cent by air. It decreased with depth to 39 per cent at 4 feet, where 19 per cent was occupied by water and 20 per cent by air.

10. Pore space in the surface 6 inches of Wabash clay loam occupied 60 per cent of the soil volume. On an average 32 per cent was filled with water and 28 per cent with air. It decreased regularly with depth (except for buried surface layers) to about 51 per cent in the seventh foot, where water occupied 41 per cent and air only 10 per cent.

11. The organic matter and nitrogen content of the roots in the upland soil were somewhat higher than in the lowland, especially in the surface 6 inches.

12. The composition of the rhizomes in the lowland was determined separately; their organic matter and nitrogen content were greater than in the roots, owing to the storage of food reserves.

13. The percentage of organic matter and nitrogen in each layer of the soil was determined, and the weight of each per square meter of soil calculated.

14. The ratio of soil organic matter to nitrogen varied in the Lancaster loam from 20 in the surface soil to 8.3 in the fourth foot, a variation typical of upland prairie soils.

15. The ratio of organic matter to nitrogen in the Wabash clay loam showed variations corresponding to the variations in the organic content at different depths. This indicates that the strata

of high organic content had accumulated a surface type of organic matter during intervals in the silting process by which the present soil has been built up.

16. Except in the surface 6 inches of soil, there is an approximately linear relation between the amount of root material and the amount of soil organic matter in the various soil horizons.

17. In the surface soil the presence of a large amount of living rhizome and root material and the favorable conditions for the decomposition of dead organic matter increase the proportion of roots and rhizomes to soil organic matter.

18. Roots and rhizomes constitute about one-tenth of the total organic matter in the surface 6 inches of soil; in the deeper sections the proportion decreases gradually from 3 to 4 per cent in the second 6 inches to 1 per cent in the fourth foot of the Lancaster soil, and 0.25 per cent in the seventh foot of the Wabash soil.

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ALTERNATION OF GENERATIONS IN ECTOCARPUS SILICULOSUS[1]

GEORGE F. PAPENFUSS

(WITH PLATES VI, VII AND THIRTEEN FIGURES)

Introduction

After the publication of the paper by BERTHOLD (**2**) in 1881, the plurilocular sporangia of *Ectocarpus siliculosus* Dillw. were regarded as gametangia and their zoids as gametes. Many investigators (**21–24, 9–12,** and others) found, however, that the zoids from the plurilocular sporangia of this species do not consistently function as gametes but more frequently act as zoospores. In order to explain this asexual behavior, the theory was generally advanced that the gametes had lost their sexual power and germinated parthenogenetically. The cytological work of KNIGHT (**7**) has proved this theory to be incorrect, for it has shown that there are two kinds of plurilocular sporangia in *E. siliculosus:* one type occurring on haploid plants and forming gametes and the other type occurring on diploid plants and giving rise to diploid zoospores which germinate into other diploid plants.

KUCKUCK (**9**) and REINHARDT (**20**) found that the zoids from the unilocular sporangium of *E. siliculosus* are zoospores while KNIGHT (**7**) believes that they function as gametes at Port Erin. The cytological studies of KNIGHT showed all the plants in this locality to be diploid. No reduction divisions occur in the plurilocular sporangium, and the zoids from this organ are diploid zoospores which germinate directly into other diploid plants. In the unilocular sporangium, on the contrary, the first division of the nucleus is a reduction division and the zoids formed are haploid. These zoids conjugate to form zygotes which develop into diploid plants. At Port Erin, therefore, no alternation of generations occurs; all the plants are diploid.

KNIGHT (**7**) studied *Ectocarpus* at Naples also, and confirmed the observations of BERTHOLD (**2**), OLTMANNS (**18**), and HARTMANN (**5**) that the zoids from the plurilocular sporangium are gametes in this

[1] Botanical contribution from The Johns Hopkins University, no. 127.

locality. The cytological work of KNIGHT showed, moreover, that the plants at Naples are haploid. Diploid plants are reported to be absent here. Since no reduction division occurs at the germination of the zygote in any brown alga thus far investigated, it is very probable that the zygotes at Naples develop into diploid plants.

KYLIN (**13**) in 1918 predicted the discovery of an alternation of generations in this species, and the cytological work of KNIGHT (**7**) suggests that it may occur in certain localities. It thus becomes important to know what rôle is played by the zoids from the unilocular sporangia on diploid plants, in all places where, as at Naples, the zoids from the plurilocular sporangia of haploid plants serve as gametes. Do the zoids from the unilocular sporangia in such localities act as gametes, as at Port Erin, or are they zoospores? It would be of great interest if they should function as gametes for it would show that this species has an alternation of generations and that both generations are capable of forming gametes, a unique condition among plants. Furthermore, may unilocular sporangia occur on haploid plants; and, if so, what is the function of the zoids?

It was with these problems in mind that the writer took up this study. The preliminary observations were made on plants found floating near Annapolis in the Chesapeake Bay during April, 1930. These plants bore only plurilocular sporangia and the zoids were zoospores. During the late autumn of 1930, material was collected at Cold Spring Harbor, Long Island, New York. This material also contained only plurilocular sporangia and the zoids were again asexual.

In order to obtain more abundant material than was found in the upper Chesapeake Bay, and to secure if possible plants with unilocular sporangia, this study was continued at Woods Hole, Massachusetts, over a period of three years. A brief preliminary account of the results obtained has been published (**19**). The present paper gives a more complete account of the functions of the different kinds of zoids, of the cytology of plants from the sea, and of the results obtained from cultural studies.[2]

[2] The following two papers on the life history of *E. siliculosus* appeared after this paper had been sent to press: FÖYN, B., Über den Lebenscyklus einiger Braunalgen. Bergens Museums Årbok. Nat. **2**:1–9. 1934; SCHUSSNIG, B., and KOTHBAUER, E., Der Phasenwechsel von *Ectocarpus siliculosus*. Österr. Bot. Zeitsch. **83**:81–97. 1934.

Observations on living marine plants

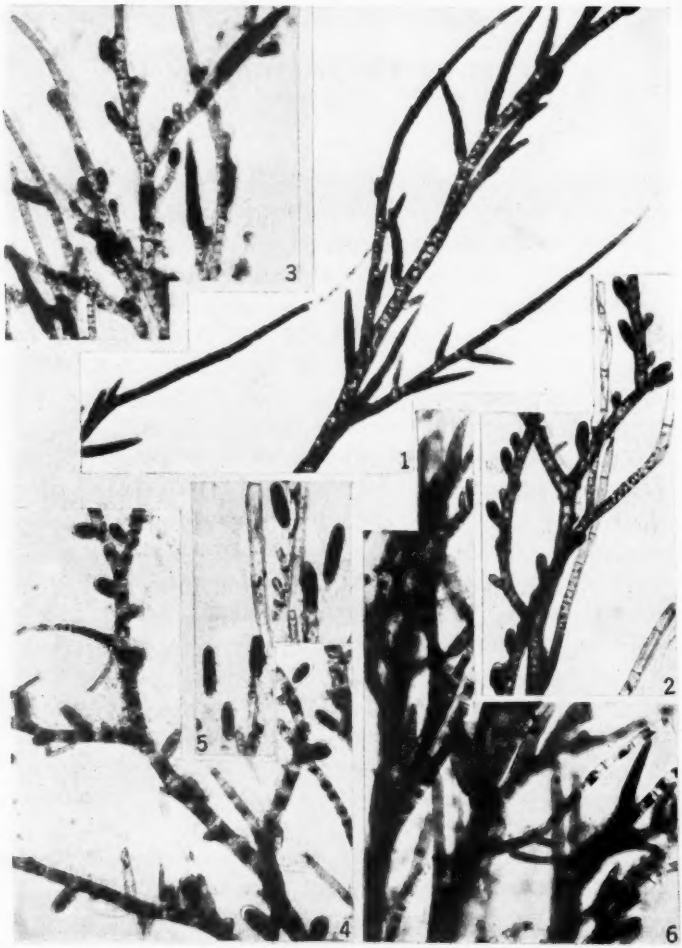
A. Function of zoids of asexual plants

All the observations on the function of the different kinds of zoids were made by placing pieces of *Ectocarpus* in a hanging drop and studying the behavior of the zoids liberated. A small drop of water was allowed to flow under the edge of the cover. This prevents the cover from sliding off the depression in the slide and also minimizes evaporation from the suspended drop. If it was necessary to keep a slide longer than five or six hours, the slide was placed in a damp chamber and the material in the suspended drop transferred to a fresh drop at least once a day. The water was changed usually in the morning since this is the time that liberation of zoids commonly occurs and a change of water often causes immediate liberation.

Ectocarpus siliculosus was first collected at Woods Hole during the early part of June, 1931; and was found on *Zostera* growing at Grassy Island. These plants bore only plurilocular sporangia (fig. 1) and the zoids were zoospores. Unilocular sporangia (fig. 2) were found during the third week of June on plants which were floating or which were caught on the ropes of lobster pots between Nobska Point and Nonamesset Island. The liberation of zoids from these sporangia was awaited with keen interest, for it was expected, in the light of Knight's (**7**) work, that the zoids would function as gametes. Contrary to such expectation, conjugation never occurred among the zoids from the unilocular sporangia, and the early germination of practically every individual zoid showed that they are zoospores.

Occasionally certain plants from the Nobska-Nonamesset region bore plurilocular as well as unilocular sporangia. The zoids from the plurilocular sporangia of such plants were also uniformly asexual. *E. siliculosus*, bearing an abundance of both unilocular and plurilocular sporangia (fig. 3) and growing as an epiphyte on *Chorda filum*, was collected later in the season at Nobska, Grassy Island, and Pine Island. During mid-summer about 50 per cent of the unilocular sporangia were parasitized by a chytrideaceous fungus; but this did not interfere with the study since many uninfected sporangia were to be had.

As plants of *E. confervoides* were found which also bore both unilocular and plurilocular sporangia simultaneously, a number of



FIGS. 1–6.—Unretouched photomicrographs showing: Fig. 1, branch of diploid plant of *E. siliculosus* bearing the plurilocular sporangia by which this species is characterized taxonomically. Material fixed in formalin acetic alcohol. Fig. 2, branch of diploid plant of same bearing unilocular sporangia only. Chromo-acetic-osmic acid material. Fig. 3, branch of diploid plant of same bearing plurilocular and unilocular sporangia on same individual. Formalin acetic alcohol material. Fig. 4, branch of *E. confervoides* with unilocular sporangia. Note that majority of unilocular sporangia are sessile in *E. confervoides* while they are usually stalked in *E. siliculosus*. Formalin material. Fig. 5, branch of *E. confervoides* bearing plurilocular sporangia. Formalin material. Fig. 6, branch of haploid plant of *E. siliculosus* bearing plurilocular organs which function as gametangia. Note that these are smaller than the plurilocular sporangia of the diploid asexual plants as shown in figs. 1, 3. Formalin material. All × 100.

observations were made on the behavior of the zoids of this species. The results obtained were the same as those for *E. siliculosus*, for the zoids from both kinds of sporangia were zoospores which germinated directly. Unilocular and plurilocular sporangia occurring on separate plants of *E. confervoides* are shown in figures 4 and 5.

B. Function of zoids of sexual plants

In July of 1931 *Ectocarpus siliculosus*, growing on *Chordaria flagelliformis*, was collected on the west end of Penikese Island, about 20 miles southwest of Woods Hole. These plants of *Ectocarpus* bore only plurilocular sporangia. As the material was comparatively free from diatoms, it was used for cytological work and fixed hourly during the next 24 hours. The morning of the third day after collection offered the first opportunity for studying the living plants. Although the material appeared to be in good condition, no liberation of zoids occurred and the plants finally died.

Cytological studies made the following winter showed that the plants from Penikese were haploid while the plants collected at Woods Hole proper, judging from the chromosome number in the plurilocular sporangia, were diploid. The cytology of the unilocular sporangium remained unsettled at the time.

Since no conjugation occurred between the presumably haploid zoids from the unilocular sporangia, it seemed clear that if conjugation occurred in the Woods Hole region, it must be between the zoids of the haploid plants at Penikese.

In order to determine whether the haploid plants were sexual, the writer returned to Woods Hole in August, 1932, and visited Penikese in early September. *E. siliculosus* was again found on *Chordaria* at the same locality as in the previous summer. In addition, other plants were obtained also on *Chorda* which grew mixed with the *Chordaria*. This collection presented two problems for solution: (1) Do the plants on *Chordaria* produce gametes? (2) Are the plants on *Chorda* from this locality asexual and do they bear both kinds of sporangia simultaneously, similar to those on *Chorda* at Woods Hole? If these two questions could be answered in the affirmative, it would show that an alternation of generations occurs in the life cycle of *E. siliculosus* at Penikese.

A microscopic examination showed that the plants on *Chorda* were similar to those occurring on this host at Woods Hole, in that they bore both kinds of sporangia, while the plants on *Chordaria* bore only plurilocular sporangia (fig. 6). The plants on *Chorda* liberated zoids freely the following morning and the zoids from both kinds of sporangia proved to be zoospores. It could not be determined whether the plants on *Chordaria* were sexual as no liberation of zoids occurred and the plants finally died. These plants at first appeared perfectly healthy and it was difficult to understand why no zoids were liberated, since the plants on *Chorda* which were collected at the same time and kept in similar running water aquaria liberated zoids freely.

It appeared probable that liberation of zoids might occur if the plants growing on *Chordaria* were taken directly from the ocean. On September 10, therefore, plants of *E. siliculosus* growing on *Chordaria* and *Chorda* respectively were collected at Penikese. Separate hanging-drop cultures were made of plants from each host. Pieces from several plants were placed in each culture. No liberation of zoids occurred that day but the following morning at daybreak liberation of zoids occurred in practically every drop and many conjugations were observed. The zygotes (fig. 14) were formed, however, only in the hanging drops containing plants growing on *Chordaria*. Although hundreds of zoids from both kinds of sporangia were present in the hanging drops containing plants from *Chorda*, not a single zygote was present in these drops. These observations thus showed that the haploid plants on *Chordaria* were in reality sexual while the diploid plants on *Chorda* had for two seasons proved themselves to be asexual.

An experiment performed at Cuttyhunk indicates that the previous failures to obtain liberation of zoids from the plants growing on *Chordaria* may be owing to the fact that *Ectocarpus* was left attached to the host. If *Ectocarpus* is removed from *Chordaria*, placed in dishes and the water changed twice a day, it liberates gametes and remains healthy for several days; while plants which are attached to *Chordaria* and kept under similar conditions fail to liberate gametes after the second day. *Chordaria* when kept in an aquarium exudes a gelatinous substance which may have a pathological effect on *Ectocarpus* and thus prevent the liberation of zoids.

The early part of October, 1932, was spent at Penikese in order to study the following questions with respect to the sexual plants: (1) Are the plants monoecious or dioecious? (2) Do unilocular sporangia occur on haploid plants? (3) Do the sexual plants have certain plurilocular sporangia which produce asexual spores and not gametes? (4) Are either or both female and male gametes capable of parthenogenetic development?

To determine whether the sexual plants are monoecious or dioecious, single branches were cut off under a dissecting microscope, transferred to a drop of water in a watch glass, and reexamined to make certain that only one branch was included. After a single branch had been obtained, it was washed in filtered sea water and cut into two pieces. One of the pieces was then placed in a drop of filtered sea water on a cover glass and a hanging-drop culture designated *A* was made of it. In a similar way a second hanging-drop culture *B* was made of one of the two pieces of a branch from another plant. The remaining two pieces of the respective branches were combined in a culture *AB*. In this way three hanging-drop cultures, *A*, *B*, *AB*, were obtained from two branches of separate plants of *Ectocarpus*.

In order to guard against the possible presence of stray gametes in the hanging drops, the cultures were made in the late afternoon or evening after liberation of gametes had ceased for the day. Each slide was furthermore examined under a compound microscope to insure that no gametes were present.

Cultures made in this manner gave conclusive results, for it was observed that zygotes were formed each morning only in the hanging drops which contained pieces from two separate plants, and in no instance did zygotes occur in the drops containing a piece from but one plant. It was thus decisively established that the sexual plants are dioecious. By transferring the small pieces to fresh hanging drops once or twice a day, it was possible to obtain liberation of gametes from some branches for several days.

In certain of the suspended drops containing two pieces from separate plants of *Ectocarpus*, zygotes were not formed although gametes were present. The absence of zygotes in such drops was rarely due to the fact that only one of the two pieces had liberated gametes but usually because both pieces were of the same sex. If

zygotes were formed in a drop on one day but not on another, it was proof that only one of the associated pieces had liberated gametes in the latter case. Since the female gametes can be distinguished from the male gametes by the short period of motility of the former, it is a simple matter to determine whether the absence of zygotes in a hanging-drop culture containing two pieces from separate plants is due to the pieces being of the same sex. Furthermore, a microscopic examination of the gametes in the two drops which contain the other half of the respective branches will show that the gametes in both drops are of the same sex. The sex of the gametes in a drop containing pieces of two branches from separate plants can be determined also by experiment as follows: Gametes were present in the cultures *G*, *GH*, and *H*, but none of these contained zygotes. The gametes were all female, judging from the short period of motility. The following morning gametes were again liberated in the three respective cultures and the gametes from a branch *E*, which had previously shown itself to be male, were added by means of a small pipette to the cultures *G* and *H*. After a few minutes, conjugation commenced in these two drops while no fusions occurred in the drop *GH*. Thus it was conclusively proved that the gametes from the branches *G* and *H* were female.

From the writer's observations there is no indication of a "relative sexuality" such as HARTMANN (5) found at Naples, but the evidence at hand is not sufficient to preclude the possibility of its occurrence.

The assembling of large numbers of male gametes around a female as figured by BERTHOLD (2) was not observed. In no instance did more than about six male gametes gather around a female simultaneously, and in many cases the female seemed to attract only one male. Conjugation was not observed to occur while a female gamete was still swarming but always took place after the female had become attached at the edge of the drop toward the source of light.

The actual fusion of gametes is a rapid process which at most does not last longer than 20 seconds; and the successive stages of conjugation of any two gametes, as are figured by BERTHOLD (2), follow each other so rapidly that they cannot be observed in the living state. But it was found that the process of fusion is greatly retarded if male gametes are added to a culture of female gametes which has been

liberated for about one hour. After this lapse of time, a firmer membrane has been formed around the female gamete, and as a result the coalescence of conjugants is accomplished more slowly. Under these conditions a male gamete may attach itself to a female gamete, perform the usual vibratory movements, and then suddenly move toward the latter as in normal conjugation; but owing to the thickened membrane of the female a coalescence does not occur and the male gamete immediately settles down motionless beside the female. It is evident that the chances of fertilization are greatly reduced by the thickening of the membrane which renders the female gamete incapable of fertilization not long after liberation. It is not known how long after liberation a male gamete remains functional.

The male gamete usually unites with the broad posterior end of the female, as was observed by BERTHOLD (2) and KUCKUCK (12). This is undoubtedly due to the fact, as BERTHOLD and KUCKUCK state, that the attached anterior end of the female is not accessible to the male.

There is considerable variation in size among the gametes of each sex, some being nearly twice as large as others. Large and small gametes arise from different gametangia respectively. At first it was thought that these large zoids were zoospores which serve to propagate the haploid generation; but it was later observed that the larger as well as the smaller zoids from the sexual plants function as gametes. In consequence zygotes of various sizes are formed: large, small, and intermediate ones, depending upon the conjugation of two large gametes, of two small gametes, or of a large with a small gamete.

Unilocular sporangia were not found on the sexual plants, and the only means by which these plants can propagate themselves is by the parthenogenetic development of the gametes of either sex, as will be discussed later.

C. MISCELLANEOUS OBSERVATIONS ON ASEXUAL AND SEXUAL PLANTS

MORPHOLOGY.—Asexual plants are more robust than sexual plants (fig. 15) and frequently attain a length of more than 7 cm., while the largest sexual plants collected were but 4 cm. long. The

cells of asexual plants are considerably larger than those of sexual plants and measure on an average $50\times70\mu$ while the cells of sexual plants average only $44.5\times45.5\mu$. The primary difference between the cells of asexual and sexual plants is thus in the length of the cells. The breadth of many cells of the sexual plants exceeds the length, a condition less common in the asexual plants. The plurilocular sporangia of asexual plants (fig. 3) are considerably larger and vary more in size and form than those of sexual plants (fig. 6). The familiar ropelike intertwining of the main axes is also more characteristic of the robust asexual plants.

DISTRIBUTION.—Sexual plants are of limited distribution and were found only at Penikese Island where they occur in large quantities and exclusively on *Chordaria*. Asexual plants are more widespread and were collected on *Spartina* and *Chorda* at Woods Hole, on *Chorda* and *Chordaria* at Cuttyhunk, and on *Chorda* at Penikese. It is a striking fact that the asexual and the sexual plants grow side by side at the latter locality but are each confined to its own host.

DISCHARGE OF ZOIDS.—The liberation of zoids, especially of the sexual plants, occurs primarily during the early morning and forenoon; but if the plants are kept in darkness overnight and placed in fresh sea water when exposed to daylight, they can often be induced to liberate zoids at other times of the day. As a rule, the opening of the plurilocular sporangium occurs at the apex. The opening of the unilocular sporangium is always at the apex.

It was not observed that the content of the unilocular sporangium is expelled in bulk as was found by KNIGHT (7). The zoids are contained in a gelatinous mass which oozes out of the sporangium, carrying the zoids with it. As the mass increases in size on the exterior of the sporangium, stages such as are shown in KNIGHT'S figures 33–35 may be observed. In many instances the perforation is very small and each zoid becomes constricted to a dumb-bell shape as it passes through the pore. After liberation, the zoids remain in a spherical mass at the apex of the sporangium for a short time, then become free and swim off individually. The process of liberation frequently consumes more than two minutes and at times some of the escaped zoids have become motile while others are still within the sporangium. These observations on the liberation of zoids from

the unilocular sporangium were made on plants cultured in aquaria, and it is possible that this environment altered the normal mechanics of discharge.

As many as thirty-two zoids were counted from a unilocular sporangium of a plant from the sea while counts of four, eight, and sixteen zoids were made from the unilocular sporangia of plants grown in aquaria. The unfavorable environmental conditions of an aquarium have a dwarfing effect on the plants and their reproductive organs. It is doubtful whether unilocular sporangia from the sea contain fewer than thirty-two zoids and it is possible that as many as sixty-four may be formed in certain very large unilocular sporangia.

Period of motility of zoids.—Female gametes have the shortest swarming period of all the zoids, and frequently become stationary at the edge of a hanging drop five minutes after liberation; about 15 minutes is their maximum period of motility. Male gametes, on the contrary, have the longest swarming period of all the zoids and at times are still motile eight hours after liberation. Zoids from the plurilocular sporangia of asexual plants often remain motile for 3–5 hours while the zoids from the unilocular sporangia seldom remain motile for more than 30 minutes.

Phototactic response of zoids.—Although the great majority of all zoids are positively phototactic, a certain proportion react either negatively or as "neutrals." No adequate explanation can be given for this difference of response, but certain observations suggest that a disturbance of the zoids during their swarming period may stimulate them to become negatively phototactic. If positively phototactic zoids are taken from a vessel and placed in a hanging drop, a large proportion of them become negatively phototactic. (Kylin (**14**) has also found this to be true.) If, however, hanging-drop cultures are made in the evening from pieces of *Ectocarpus* and these cultures placed in a dark room with light entering only through a small window, practically 100 per cent of the zoids liberated the following morning are positively phototactic.

Size of zoids.—A considerable difference in size exists among the three types of zoids. The zoospores from the unilocular sporangium are large and measure on an average $9.1 \times 18 \mu$; the gametes are small

and measure on an average only $4\times7.4\ \mu$; and the zoospores from plurilocular sporangia of asexual plants are intermediate in size and measure on an average $4.8\times9.5\ \mu$. Large zoids from unilocular sporangia and smaller zoids from diploid plurilocular sporangia are shown in figures 16–18. The difference in size between the gametes and the asexual zoids may be seen in figures 14, 16, 17, and 18.

SEASONAL CYCLE.—Although the writer's data regarding the seasonal cycle of *E. siliculosus* are not entirely complete, certain facts are worth recording. At Woods Hole, in contrast to the several localities where previous investigators have worked, unilocular sporangia are common and can be found throughout the summer months. Plants bearing unilocular sporangia were collected for the first time during the third week of June, 1931; but since these plants contained many mature and empty unilocular sporangia, there is reason to believe that these organs had begun to appear earlier in the year. Unilocular sporangia evidently disappear in autumn at Woods Hole and remain absent during the winter. Plants collected at Woods Hole on October 30, 1931, bore very few of these organs but many plurilocular sporangia. (*E. siliculosus* was rare at this time of the year.) The latest collection of the year was obtained from a small amount of material received from Woods Hole on December 21, 1931. These plants bore many plurilocular sporangia similar to those shown in figure 1 but had no unilocular sporangia. Although unilocular sporangia were abundant on *Ectocarpus* at Penikese during the early part of September, 1932, they had disappeared completely by the first week of October of that year. During the latter half of September, 1933, unilocular sporangia were found at Woods Hole on two occasions only; and they were entirely absent on plants received from Woods Hole on November 27, 1933.

The evidence from three seasons' collecting thus indicates that unilocular sporangia occur in the Woods Hole region primarily during the summer months while diploid plurilocular sporangia occurred at all seasons at which collections were made (June 5 to December 21). It is possible that the asexual plants occur throughout the year at Woods Hole and that their rareness during winter is owing to the transitory habit of the hosts.

The available data on the seasonal occurrence of sexual plants

are too fragmentary to justify any conclusions. Plants with gametangia were first collected in July, 1931, again in September of 1931 and of 1933; and they were still abundant during the first two weeks of October, 1932.

Cytology

FIXING.—The following fixing agents gave good results: (1) CHAMBERLAIN'S (3) chromo-acetic-osmic acid solution which consists of 97 parts sea water, 1 part chromic acid, 1 part glacial acetic acid, and 1 part of 1 per cent osmic acid. The time of fixation required is four to five minutes. (2) Allen's modifications of Bouin's fluid (17): (a) B-15, which is the original Bouin's fluid made up with sea water to which is added 2 parts of urea and 1.5 parts of chromic acid; (b) B-3, which consists of picric acid, saturated solution in sea water, 75 parts; formalin 15 parts; glacial acetic acid 10 parts; and chromic acid 1 part. The time of fixation required is one to two hours. Mitoses are more abundant if the material to be fixed is first kept in darkness for two or three hours, as is suggested by DAMMANN (4).

DEHYDRATING.—Taylor's method (17) for dehydration was followed with minor modifications. The material was left one-half to one hour in each grade of the alcohol, salt water, and fresh water, indicated in table I, and was allowed to remain in 70 per cent alcohol until dehydrated for imbedding.

In making paraffin sections, the trouble caused by epiphytic diatoms may be partly overcome if the material to be sectioned is treated for 24 hours with a 5 per cent solution of hydrofluoric acid in 70 per cent alcohol, and subsequently washed in 70 per cent alcohol.

SECTIONING AND STAINING.—The usual methods of clearing, infiltration, and imbedding were followed. By placing the material selected for sectioning in a small cloth bag and transferring the bag from grade to grade of the alcohols and xylols, much time is saved. The material is taken out of the bag when it is in the final xylol. Rubber tubing rings placed on glass slides coated with glycerine, as described by BAUMGARTNER and WELCH (1), were used as imbedding troughs.

The sections were cut 3-4 μ thick. Since a few diatoms always remain attached to the material, a great deal of time lost in sharpening the knife can be avoided by using Wade and Butcher safety blades and the microtome blade holder of the same make.

The material fixed in chromo-acetic-osmic acid was bleached in a 5 per cent aqueous solution of hydrogen peroxide for one hour.

Feulgen's reaction (LEE **15**, pp. 306–307, 437–438; MARGOLENA **16**; KRAUSE **8**, p. 1792; WESTBROOK **28**) and Haidenhain's iron-alum

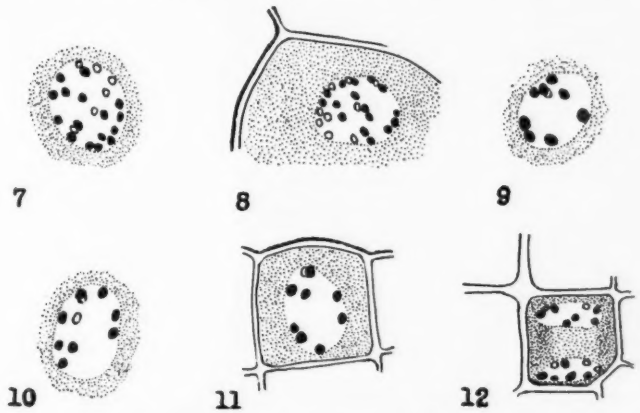
TABLE I

WATER, FRESH (PARTS)	WATER, SALT (PARTS)	ALCOHOL, 95 PER CENT (PARTS)
1	93	1
2	91	2
3	89	3
4	87	4
5	85	5
6	83	6
7	81	7
8	79	8
9	77	9
10	75	10
$12\frac{1}{2}$	70	$12\frac{1}{2}$
15	65	15
20	$57\frac{1}{2}$	$17\frac{1}{2}$
25	50	20
30	40	25
35	30	30
40	15	40
45	.....	50
35	.....	60
25	.....	70

haematoxylin were used as stains, but Feulgen's reaction gave the more satisfactory results. All the mitotic figures are drawn from preparations stained by this method. The chief advantage of Feulgen's reaction lies in the fact that, with correct treatment, it is practically specific for chromatin; and it leaves nucleoli and cytoplasmic inclusions unstained. The cytoplasm appears perfectly homogeneous and clear, especially if the material is fixed with B-15 or B-3. In this study the sections were treated with the 0.5 normal HCl for ten minutes and stained in the fuchsin sulphurous acid for four hours.

CHROMOSOMES.—The primary objective of this phase of the work was to determine whether the chromosomal cycle coincides with the

morphological phases of the life cycle. Although the results are incomplete in certain details, it has nevertheless been possible to establish that: (1) The asexual plants, with both plurilocular and unilocular sporangia, are diploid. No chromosome reduction occurs in the plurilocular sporangium and the nuclei in this organ contain about sixteen chromosomes (figs. 7, 8). In the unilocular sporangium on the contrary, one of the early nuclear divisions is undoubtedly a reduction division, for the nuclei which are formed later contain but



FIGS. 7–12.—Allen's fixative; Feulgen's reaction. Camera lucida drawings from paraffin sections: Fig. 7, polar view of early anaphase in plurilocular sporangium of asexual plant showing at upper level diploid number of chromosomes (16) and at a lower level 6 (unshaded) chromosomes of set for opposite pole. Fig. 8, anaphase from another plurilocular sporangium showing 16 chromosomes at upper level and 9 (unshaded) chromosomes of set for opposite pole. Fig. 9, polar view of metaphase in a unilocular sporangium showing haploid number of chromosomes (8 or 9), eight at upper level and one at lower level. Fig. 10, similar view, but from a different unilocular sporangium, showing haploid number of chromosomes (8) at upper level and two additional ones, probably of set for opposite pole, at lower level. Fig. 11, polar view of metaphase in a plurilocular sporangium of sexual plant showing haploid number of chromosomes (8 or 9); one is at a lower level than the other eight. Fig. 12, profile view of late anaphase or early telophase in a plurilocular sporangium of another sexual plant, showing haploid number of chromosomes (8) at each pole. All ×4320.

eight or nine chromosomes (figs. 9, 10). (2) The sexual plants are haploid, the nuclei in the plurilocular sporangia of these plants containing eight or nine chromosomes (figs. 11, 12). Cytological obser-

vations thus show that there is an alternation of generations in the life cycle of *E. siliculosus* growing in the region of Woods Hole.

Cultures

A. AQUARIUM CULTURES.—Two-liter battery jars were employed as aquaria, and these aerated by the aid of aspirators. The aquarium was kept cool by placing it within a larger jar and allowing the outflow of water from the aspirator bottle to run into the latter. Figure 13 illustrates the aerating system. The filter pump may be attached either to the faucet or, as in this case, to the aspirator bottle. Care has to be exercised not to place too much material in an

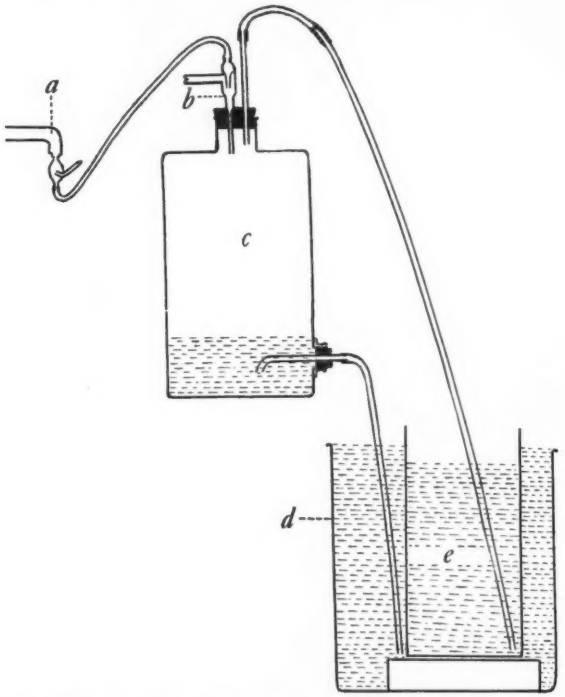


FIG. 13.—Diagram of apparatus for aerating an aquarium: *a*, faucet; *b*, filter pump; *c*, aspirator bottle; *d*, fresh water jar; *e*, salt water aquarium.

aquarium of this size. The best results are obtained by employing several aerating systems. As the sea water evaporates from the aquarium, distilled water should be added to prevent the sea water from becoming too saline.

By thus aerating and cooling the aquarium, it was possible to grow *Ectocarpus* for two months in the laboratory at The Johns Hopkins University; and the plants undoubtedly would have survived for a longer time if small crustaceans had not devoured them. Reproductive organs continue to be formed on plants growing in aquaria but they as well as the newly formed branches become dwarfed after a short time.

It was found that many unilocular sporangia are formed on asexual plants kept in aquaria. Plants collected at Woods Hole on October 30, 1931, bore but few unilocular sporangia and many plurilocular sporangia. After a week in the aquaria, these plants started to produce many unilocular sporangia while the plurilocular sporangia decreased in number, and after 14 days these plants bore decidedly more unilocular than plurilocular sporangia. Likewise, asexual plants which were collected at Penikese in October, 1932, and which contained no unilocular sporangia, started to produce these organs after they had been in an aquarium for two weeks. Sexual plants collected at the same time and kept in a similar aquarium failed to produce unilocular sporangia, a fact which gives additional evidence that unilocular sporangia are organs confined to the diploid asexual generation, as already discussed.

B. SILICA-GEL CULTURES.—Silica gel (**26, 27**) is a medium which deserves to be used more generally in the culturing of algae. The inorganic composition of this gel renders it superior to all other solid media in that it supplies no nourishment to the bacteria which are inevitably introduced into a medium with the spores of the alga to be cultured.

The following simple method for preparing the gel was shown to the writer by the Reverend A. KEEFE: Equal parts of sodium silicate, sp. gr. 1.1, and hydrochloric acid, sp. gr. 1.09, are mixed by stirring and the solution poured into petri dishes. The dishes are allowed to remain undisturbed for several hours, or preferably overnight, for the gel to set, then placed in a vessel and washed in running

fresh water for at least 24 hours to remove the chlorides. In order to have the gel saturated with sea water the dishes are left in a vessel containing filtered sea water for about 36 hours. The plates are then ready for inoculation or may be autoclaved before they are used.

It is necessary to add a liquid medium to silica gel plates since the gel dries within a comparatively short time. The liquid culture medium employed so successfully by SCHREIBER (**25**) for the culturing of *Laminaria* gametophytes was used. This liquid medium consists of:

$NaNO_3$	0.1 gm.
Na_2HPO_4	0.02 gm.
Distilled H_2O	50.0 cc.
Sea H_2O	1000.0 cc.

Although plants have been raised from the spore to the formation of reproductive organs by this method, the writer has not yet obtained the entirely satisfactory results which may be expected from further experiment with this culture medium. A major source of trouble has been the rapid multiplication of diatoms which are introduced into the plates with the zoids.

C. HANGING-DROP CULTURES.[3]—The best cultural results were obtained from hanging-drop cultures. This method of culturing zoids is similar to the one already described for the observations on the behavior of zoids, with the exception that the parent plants are removed from the drops after liberation of the zoids has occurred and the slides are then placed in a damp chamber. The attached zoids adhere firmly to the glass, and it is possible to wash the covers by squirting sea water on them from a pipette, and thus rid them of most of the diatoms which are introduced with the parent plants.

It is necessary to dry the slides and covers frequently with filter paper, since the condensed vapor which accumulates on them tends to float up the cover slips and to flow into the hanging drops. Since the young plants adhere to the cover glass, it is possible to withdraw the drop of water with filter paper and replace it with fresh sea water from time to time without danger of losing the plantlets. In many cases the drop of sea water was replaced with SCHREIBER'S (**25**) culture fluid. Although diatoms multiplied rapidly in the silica gel

[3] A more satisfactory method for culturing algae is described by KYLIN (**14**) in a paper which appeared after this study had been completed.

cultures they never constituted a serious problem in the hanging-drop cultures. By the hanging-drop method plants were grown successfully from diploid zoospores, zygotes, parthenogenetic gametes, and haploid zoospores.

Diploid zoospores usually germinate within two to five hours after they have become attached. A series of early developmental stages of plants from these zoids is shown in figures 26–32. The plants shown in figure 19 have formed plurilocular sporangia. These plants previously also bore unilocular sporangia but unfortunately no photograph was taken of these earlier sporangia. In one case mature plurilocular sporangia were present on a 45-day old plant. In the majority of cultures, however, the young plants did not produce reproductive organs within the first two months. The F_1 generation of plants from diploid zoospores is shown in figure 20. Plants grown in hanging-drop cultures are small but otherwise appear perfectly normal. Some of the plants were still in perfect condition when six months old.

The zygotes occasionally begin to germinate after nine hours but usually not before two or three days. The two eye-spots are often still visible after 48 hours. The sporelings from these structures develop very slowly as contrasted with those from diploid zoospores.

Only about 5 per cent of the unfused male and female gametes develop parthenogenetically. In a few cultures about 50 per cent of the unfertilized gametes germinated and it is possible that in the ocean considerably more than 5 per cent of the unfused gametes develop parthenogenetically. The parthenogenetic gametes are very slow in germinating. Occasionally the germ tube is discernible after 24 hours but more commonly it is 36 or 48 hours, or even more, before germination begins. The unfused gametes frequently enlarge to about twice their original size before they germinate.

The haploid zoospores from the unilocular sporangium usually begin to germinate within two to three hours after liberation. After eight hours the germ tube may be one-half the length of the zoid and after 24 hours the sporeling may have become a two-celled filament. Considerable variation in the rate of development exists, however, between individual sporelings. A series of developmental stages of plantlets from haploid zoospores is shown in figures 21–25.

After the cultures of plants from zygotes, parthenogenetic male

and female gametes, and haploid zoospores had been grown for two months without fruiting, they were accidentally allowed to dry up. Well developed vegetative plantlets were obtained from these cultures, however, and it can be concluded that zygotes, parthenogenetic gametes, and haploid zoospores, as well as the diploid zoospores, all play an active rôle in the life cycle of *E. siliculosus*.

From the observations on germination, it is evident that the initial growth of a sporeling always occurs from a germ tube arising at the narrow anterior end of a zoid, and after this tube has become several-celled, a second filament arises at the posterior end of the zoid (figs. 24, 25). A zoid always becomes attached in such a way that the narrow end is directed toward the incident light; and it seemed possible that the origin of the germ tube at this end of the zoid may be due to a photic stimulus. In order to test this, hanging-drop preparations were allowed to liberate zoids in a dark room lighted only from a very small window. After the zoids had settled at the edge of the drop toward the light, each with its narrow anterior end directed toward the window, the slides were turned through 180° and placed in a damp chamber in front of the window. Under these conditions the anterior end of the zoids, now directed away from the light, still continued to give rise to the initial germ tube. The position of the germ tube is thus independent of the direction of the incident light. Whether the place of origin of the germ tube is determined by a contact stimulus or by the polarity of the zoid is not known. In zygotes the germ tube arises from the anterior end of the female gamete. The further development of the small plants shows that they are positively phototropic, however, for the majority of filaments bend and grow toward the light. Figure 33 shows part of a hanging-drop culture treated in this way. The majority of filaments are growing toward the light and away from the edge of the drop. Figure 34, from the same culture but taken at the edge of the drop which is toward the light, shows that practically every filament is growing toward the light.

Discussion

The results of this investigation show that there is an alternation of generations in the life cycle of *Ectocarpus siliculosus* in the region

of Woods Hole. The observations of three summers have shown, however, that there is a marked difference in the distribution of the two generations. Both generations grow at Penikese Island, 20 miles from Woods Hole, while only asexual plants occur at Woods Hole itself. Since large numbers of haploid zoids from unilocular sporangia are present at Woods Hole, the absence of sexual plants in this locality becomes a matter of considerable interest. The explanation of this condition was not forthcoming until the sexual plants were found at Penikese and it was observed that they are obligate epiphytes on *Chordaria flagelliformis*, an alga which does not occur at Woods Hole. The environment at Woods Hole is unfavorable for *Chordaria*, which usually grows in exposed localities like the one at Penikese.

The asexual plants of *Ectocarpus* occur at Penikese only on *Chorda filum* which grows mixed with *Chordaria*. This would seemingly indicate that the asexual plants are obligate epiphytes on *Chorda*. This is not the case, however, for these plants occur also on *Spartina* at Woods Hole and were found on one occasion on a small amount of *Chordaria* collected at Cuttyhunk Island.

It is not known whether the condition in the region of Woods Hole also applies to other localities, since the previous investigators, who found the sexual plants of *E. siliculosus*, do not specify the host. The dependence of the sexual generation on a specific host is not unique for *Ectocarpus*, however, but is also true for *Pylaiella litoralis*. KNIGHT (**6**) found that the diploid plants of *Pylaiella* occur on both *Fucus* and *Ascophyllum* while the haploid plants are confined to *Ascophyllum*, even when it grows mixed with *Fucus*. (KYLIN (**14**) found that the haploid plants of *Pylaiella* do not grow directly on *Ascophyllum* but on *Sertularia pumila* which is attached to the latter.) It is possible that the dependence of the sexual generation on a certain host is a phenomenon of more common occurrence in algae than has been recognized.

The absence of the sexual generation of *Ectocarpus* in certain localities is due, undoubtedly, to an effect of the environment which inhibits the formation of unilocular sporangia. This is the case, apparently, at Kristineberg, Sweden, where there are only asexual plants which propagate themselves by means of zoospores from the

plurilocular sporangia (14). It is probable that a very slight difference in the environment may determine the absence of unilocular sporangia, for the writer found that asexual plants growing on *Spartina* at Grassy Island bore plurilocular sporangia only, while the plants which grew on *Chorda* in the same locality but in slightly deeper water bore both plurilocular and unilocular sporangia.

Since *Ectocarpus* has an alternation of generations in the region of Woods Hole, we would expect this to be true in other localities also, especially at Naples where sexual plants are abundant. Although there is as yet no record of an occurrence of asexual plants at Naples (except the plantlet shown in BERTHOLD'S (2) figure 8), it is evident that such plants exist there. Both BERTHOLD (2) and OLTMANNS (18) observed "neutral" spores from plurilocular sporangia, and OLTMANNS states that these are generally larger than the gametes, facts which indicate that these zoids were diploid zoospores. It is obvious that REINHARDT (20), SAUVAGEAU (22), and KUCKUCK (12) also had both generations of *E. siliculosus* at Sevastopol, Guéthary, and Helgoland respectively.

The plants investigated by KNIGHT (7) at Port Erin are similar to those occurring at Woods Hole proper, but the zoids from the unilocular sporangium behave entirely differently in the two localities, in that they function as zoospores at Woods Hole while they act as gametes at Port Erin. It is possible that the cases which KNIGHT designated as conjugations were abnormal fusions or sister zoids that had not become separated from each other in the sporangium. The following facts suggest that KNIGHT misinterpreted abnormal structures for conjugations: (1) fusions were not abundant; (2) conjugation occurred while the zoids were motile; (3) the process of fusion required about 20 minutes; (4) at times the zoids fused in groups. In contrast to this the writer found that many conjugations occur between the gametes from the plurilocular sporangia of haploid plants; conjugation always occurs between a motile male gamete and an attached female gamete; the process of fusion requires less than one-half minute; and the gametes never fuse in clumps.

KNIGHT believes that the unfused zoids from the unilocular sporangium die at Port Erin, but judging from the results obtained

at Woods Hole it may be concluded that the great majority of these zoids are capable of development into haploid plants. The absence of haploid plants at Port Erin may be due to the absence of the essential host as is the case at Woods Hole proper.

Summary

1. An alternation of generations occurs in the life cycle of *Ectocarpus siliculosus* growing in the region of Woods Hole.

2. The diploid asexual plants bear unilocular and plurilocular sporangia on separate individuals or simultaneously on the same individual. In the plurilocular sporangium are formed diploid zoospores which germinate directly into other asexual plants.

3. A reduction division occurs in the unilocular sporangium and the haploid zoids formed in this organ germinate into haploid sexual plants.

4. The sexual plants are dioecious and produce physiologically anisogamous gametes in plurilocular sporangia. The zygotes develop into diploid asexual plants.

5. The sexual generation propagates itself by the parthenogenetic development of about 5 per cent of the unfused gametes of either sex.

6. Haploid plants are inferior to diploid plants in stature, size of cells, and size of plurilocular sporangia.

7. Plants were grown in suspended drops from spore to spore. Some cultures lived for more than six months.

8. Sexual plants were found only at Penikese Island where they occur on *Chordaria*. Asexual plants are widely distributed in the region of Woods Hole and occur on *Chorda*, *Chordaria*, and *Spartina*.

9. It is suggested that the sexual plants are obligate epiphytes on *Chordaria* and that their absence at Woods Hole proper is owing to the absence of this host in this locality.

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EXPLANATION OF PLATES VI, VII

All figures are unretouched photomicrographs of *E. siliculosus*.

PLATE VI

FIG. 14.—Zygotes (z) and unconjugated gametes (g). Note larger size and two eyespots of zygotes. Living hanging-drop culture. ×504.

FIG. 15.—Asexual (2x) and sexual plants (x). Note difference in size. Formalin material. ×7/10 natural size.

FIG. 16.—Haploid and diploid zoospores, formed in unilocular and plurilocular sporangia respectively, of diploid asexual plants. Three large zoids are from unilocular sporangium. Living hanging-drop culture. ×352.

FIG. 17.—Same but from a different culture. Ten large zoids from unilocular sporangium and two small zoids on left from plurilocular sporangium. ×352.

FIG. 18.—Same but from a different culture, 5 hours after liberation of zoids. Two of the seven large haploid zoids from unilocular sporangium have already germinated. ×332.

FIG. 19.—Plants from diploid zoospores bearing plurilocular sporangia. Living hanging-drop culture, 3 1/2 months old. ×70.

FIG. 20.—Living F_1 plantlets from zoospores of plurilocular sporangia formed on plants raised in hanging-drop culture from diploid zoospores. ×184.

FIG. 21.—Living young plants from haploid zoospores. Hanging-drop culture, 3 days old. ×344.

FIG. 22.—Living plantlets from haploid zoospores. Hanging-drop culture, 16 days old. ×344.

FIG. 23.—Same, 21 days old. ×352.

FIG. 24.—A 32-day old plantlet from a haploid zoospore. Living hanging-drop culture. ×344.

FIG. 25.—Another plant from same culture and of same age. ×344.

PLATE VII

FIG. 26.—Diploid zoospores, 10 hours after liberation: *c*, early stage in germination. Living hanging-drop culture. ×384.

FIG. 27.—Same culture, 57 hours old. ×384.

FIG. 28.—Same culture, 5 days old. ×384.

FIG. 29.—Same culture, 13 days old. ×374.

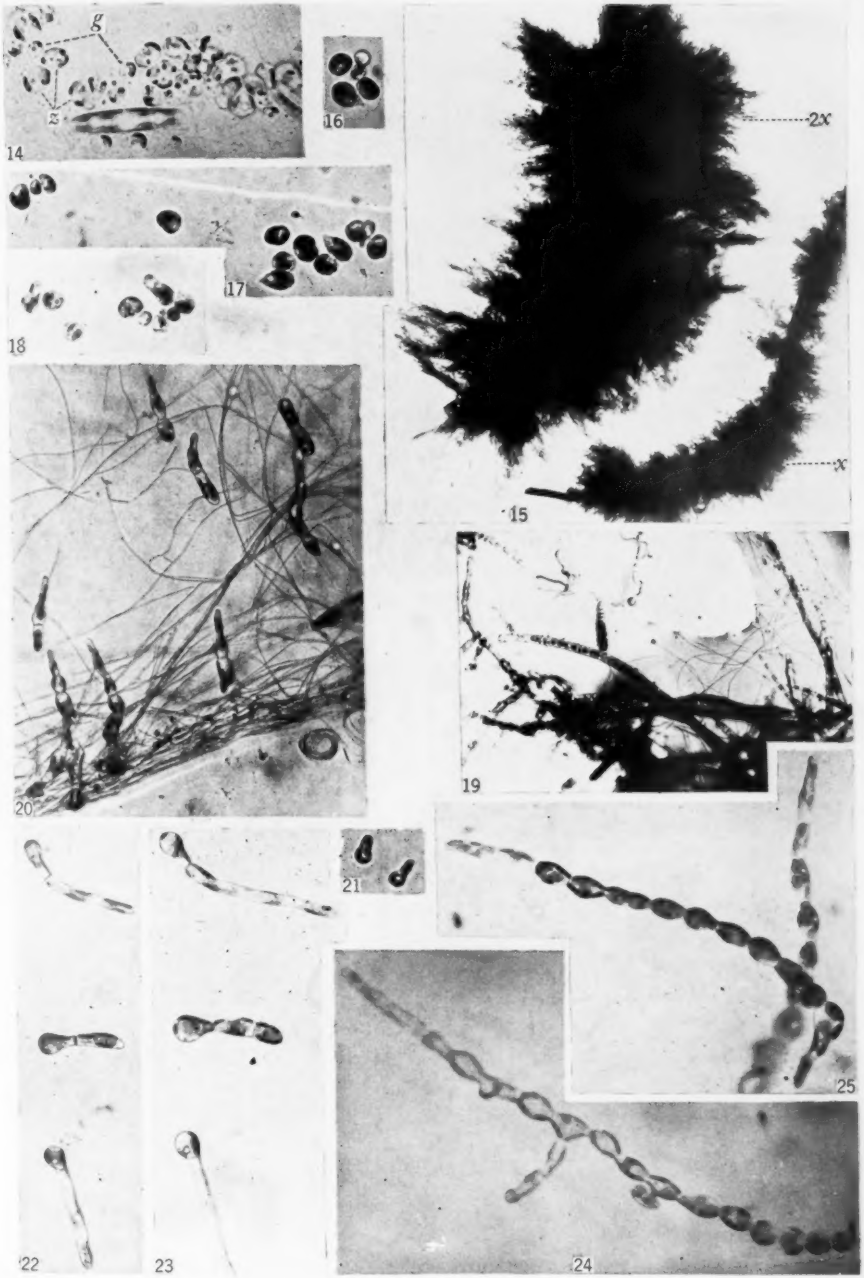
FIG. 30.—Living 14-day old plantlets from diploid zoospores. Hanging-drop culture. ×80.

FIG. 31.—Living 22-day old plantlets from diploid zoospores. Hanging-drop culture. ×378.

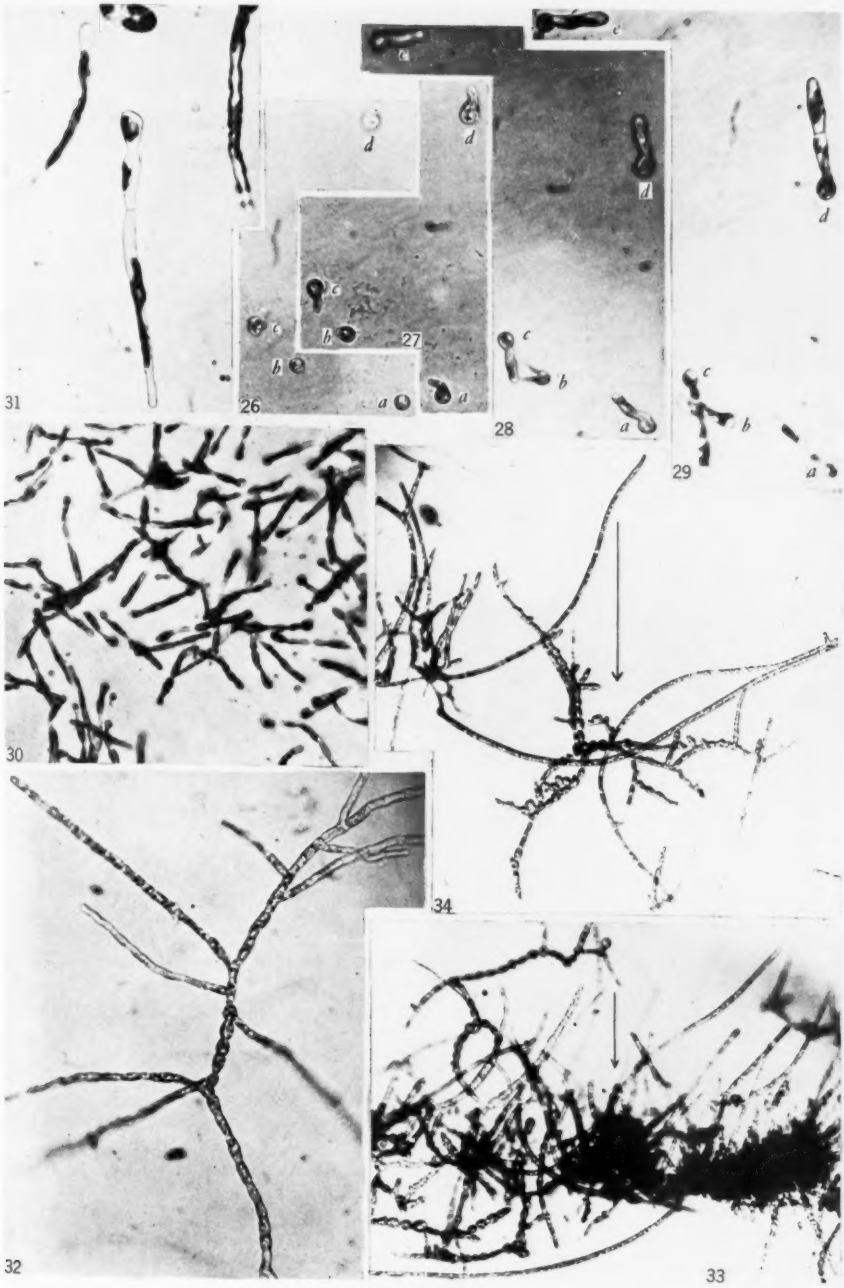
FIG. 32.—Living 30-day old plantlet from diploid zoospore. This sporeling developed unusually rapidly. Hanging-drop culture. ×152.

FIG. 33.—Darker side of a 44-day old hanging-drop culture from diploid zoospores. This culture was kept in a dark room with light entering through a small window. After the zoids had become attached at edge of drop facing window, the slide was turned through 180°. The filaments are growing away from the edge of the drop and toward the source of light (indicated by arrow). ×68.

FIG. 34.—Illuminated side of same culture. The filaments are growing toward the edge of the drop (toward source of light). ×68.



PAPENFUSS on ECTOCARPUS



PAPENFUSS on ECTOCARPUS

IMPROVED METHODS FOR THE PURIFICATION OF THE COMMON CAROTENOIDS, AND THE QUANTITATIVE MEASUREMENT OF THEIR ABSORPTION SPECTRA

ELMER S. MILLER[1]

(WITH THREE FIGURES)

Introduction

Experimental data obtained by STOKES (**15**) in 1864 suggested that plant chloroplasts contained two yellow pigments. Developing the method of partition between solvents, STOKES separated carotene and leaf xanthophyll, both of which he differentiated spectroscopically. He was not aware of the ease with which the carotenoids oxidize, and consequently his data lack much value because of the heat treatments and exposures to oxygen to which he subjected the compounds.

In 1883 BORDIN (**1**), by isolating two different types of crystals from plant extracts, demonstrated that the plant chloroplasts contain more than one yellow pigment. According to their solubilities, these crystals belonged to two different groups. To one group belonged the hydrocarbons (carotenes), which were very soluble in petroleum ether but only slightly soluble in ethanol; to the other group belonged the hydrocarbons with hydroxyl groups (xanthophylls), which were soluble in other alcohols but only slightly soluble in such solvents as petroleum ether.

After a study of the physical and chemical properties of plant pigments, TSWETT (**17**) introduced a new method for the separation of the component pigments. He filtered a moisture-free carbon disulphide (or petroleum ether) solution through a column of dry calcium carbonate, which was packed as tightly and evenly as possible. By this means a chromatogram was obtained and the pigments were differentiated into zones by virtue of this preferential adsorption of the calcium carbonate for the different pigments. By extracting these zones with suitable solvents, solutions of the different pig-

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ments were thus obtained. In addition to two chlorophylls, TSWETT succeeded in separating what he identified as several different xanthophylls. Although recent work indicates that some of TSWETT's xanthophylls were substances resulting from the oxidation of the carotenoids, his methods should be exhaustively reinvestigated. In 1909, ESCHER (**2**) developed a simple crystallization method for the isolation of carotene from carrot roots with excellent yields.

Recently KARRER (**4, 5**), KUHN (**7, 8**), and STRAIN (**16**) by chromatographic methods isolated alpha and beta carotene, but their publications do not include sufficient detail as to how the purifications were performed to make possible the confirmation of their results. Methods for the isolation of alpha and beta carotene and leaf xanthophyll are presented in this paper. Quantitative spectral analyses of binary mixtures of carotenoids have been made on pure substances thus obtained.

Experimentation

In this paper the preferential adsorption method introduced by TSWETT is combined with parts of ESCHER's method. Neither of these workers conducted their purification in an inert atmosphere, a precaution which is employed in this study. The carotenoid solutions are never heated above 48° C. The tests employed for determining the purity of the carotenoids are the melting point, the optical rotation, and the absorption spectrum. Emphasis is placed on the last method because of the accuracy with which the specific absorption coefficients at any desired wave length may be measured by a recently developed spectro-photoelectric method (**21**).

PURIFICATION OF ALPHA AND BETA CAROTENES.—The methods presented here are based on 15 purifications (15 samples of each isomer) of the components, and are not only the shortest but the simplest methods found by the writer to give complete separation of the carotene isomers. The percentage composition of all solvents used is expressed on the basis of volume.

From 120 to 125 kg. of carrots are sliced and dried at 44° to 48° C. The oven employed for this purpose is equipped with a fan to insure the flowing of a continuous stream of warm air over the carrot pulp. Thirty to 36 hours is sufficient time for complete de-

hydration. The dried carrots are ground to a fine powder in a burr mill. The powder is placed in a continuous extractor and the pigments completely extracted by petroleum ether (b.p. 60°–70°). Approximately 36 hours is required for extraction. Under diminished pressure at 45° C., the extract is concentrated to 2.5 liters. The solution after drying with sodium sulphate is filtered through a layer of calcium carbonate about 1.5 cm. thick. This carbonate filter is made in a Buchner funnel 19 cm. in diameter and 6 cm. in depth. The calcium carbonate layer is made by wetting the calcium carbonate with petroleum ether and applying suction to the Buchner funnel. As the petroleum ether is removed, the calcium carbonate is pressed down firmly until it forms a hard dry layer.

To the concentrated carotene solution, 25 gm. of potassium hydroxide is added. The saponification is carried out in a balloon flask for three hours at 45° C. The balloon flask is closed with a stopper fitted with a capillary outlet tube and a 3 mm. inlet tube which extends within 2 cm. of the bottom of the flask. During saponification, a stream of nitrogen is bubbled through the solution to insure a constant agitation and to exclude oxygen. After cooling the saponified solution, it is transferred to a 4 liter glass separatory funnel and washed six times with 500 cc. portions of distilled water. The major portion of the xanthophyll is removed from the carotene solution by washing the latter with seven 500 cc. portions of 89 per cent methyl alcohol. This fractionation is only approximate. After drying with 150 gm. of sodium sulphate, the carotene solution is filtered through another layer of calcium carbonate. The carotene solution is now a deep ruby red, and when held between the eye and a small electric filament lamp, the filament appears red and well defined. The solution is concentrated to 150 cc. under reduced pressure. The concentrated solution is now transferred to an Erlenmeyer flask, placed under nitrogen, and set in the refrigerator for 24 hours. The carotenes crystallize out with an appreciable amount of colorless impurities.

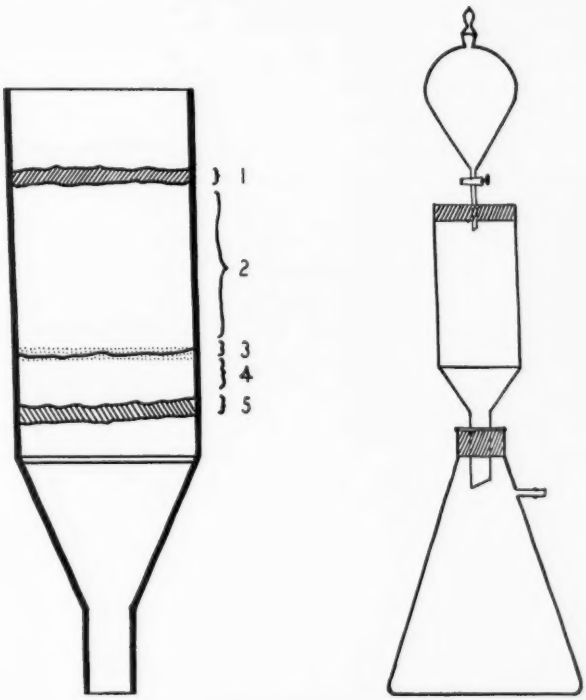
By filtration the carotene crystals are recovered on a filter paper in a Buchner funnel. After one more recrystallization from petroleum ether (b.p. 60°–70°) the crystals melt at 163° C. The yield is approximately 3 gm. (end of ESCHER's method).

The major portion of the colorless impurities are removed by further recrystallization in an inert atmosphere. The crystals (0.60 gm. sample) are dissolved in boiling cyclohexane against which a stream of nitrogen is directed. As soon as a true solution is formed, the flask containing the carotene solution is quickly stoppered and immersed in a carbon dioxide-acetone bath. Within 10 minutes the solution is a solid. The solid solution is placed on a steam bath until approximately two-thirds of it has returned to the liquid phase. In this state the major portion of the impurities are insoluble in the cyclohexane and consequently form globules.

The solution is quickly decanted into a Buchner funnel containing a filter paper. The funnel is connected to a flask to which suction is applied. A stream of nitrogen is directed against the solution, which filters quickly. The globules pass readily through the filter paper which retains the crystalline carotenes. The crystals are washed with two 30 cc. portions of petroleum ether (b.p. 30°–35°). The carotene crystals melt at 176°–177° C. Following the second recrystallization from cyclohexane as previously described, and a recrystallization from petroleum ether (b.p. 60°–70°), the melting point is raised to 181° $C_{cor.}$. The yield is 0.30 to 0.32 gm.

A carotene sample (0.31 gm.) is dissolved in the smallest possible amount of petroleum ether (b.p. 60°–70°) and filtered through a 10 cm. layer of calcium hydroxide. This layer is made like the carbonate layer. For this purpose a glass funnel 14 cm. deep and 7 cm. in diameter (fig. 1) fitted with a sintered glass filter is used. The carotene solution penetrates the calcium hydroxide layer, forming a chromatogram (fig. 1). The adsorbed carotenes are washed with 150 cc. petroleum ether which aids in effecting a more complete separation. The lower zone may approach within 1 cm. of the sintered filter. If the carotene solution is too dilute, the thickness of the calcium hydroxide layer as described is not sufficient to adsorb the carotenes, and consequently some of the alpha carotene will occur in the last portion of the filtrate. It is impossible to have the carotene solution too concentrated for a satisfactory chromatographic separation of the isomers. If the calcium hydroxide layer is much thicker than 10–12 cm., the rate of filtration is too slow. Figure 2 illustrates the set-up employed for chromatographic analyses.

The top layer (fig. 1) is of a heterogeneous nature. The color of this zone varies from a deep bright red to a grayish red. Since this layer I contains the last traces of impurities and oxidized carotenes, it is removed with a spatula and discarded. Layer II contains beta carotene and is readily recognized by its deep orange color. It is quickly



FIGS. 1, 2.—Fig. 1 (left), carotene chromatogram; fig. 2 (right), adsorption apparatus.

removed and placed in an Erlenmeyer flask containing petroleum ether. If the carotene cannot be elutriated immediately, the adsorbed carotene should be kept in a stoppered flask under an inert gas. In order to obtain layers II and IV free from contamination, it is necessary to remove an intermediate layer III which includes the line of demarcation. Layer IV is removed with the same precautions

as layer II. Layer V contains an unidentified carotenoid which is discarded.

For the best results, the respective carotenes should be elutriated as quickly as possible from the calcium hydroxide. This is readily accomplished by stirring the solutions and filtering through the same funnel that contained the chromatogram. When removing the carotene from the calcium hydroxide in layer II, it is necessary to wash the calcium hydroxide with 500 cc. of petroleum ether containing 2 per cent methyl alcohol. About 125 cc. of this solution is sufficient to remove the alpha carotene from the adsorbent in layer IV. The respective solutions of alpha and beta carotene are concentrated under reduced pressure to one-eighth their volume, cooled, and set in the refrigerator. The carotenes usually crystallize overnight (in some instances it is necessary further to concentrate the solutions).

The carotene crystals are recovered in a Berlin crucible by filtration. KARRER (5) reports that a single filtration through a column of calcium hydroxide is sufficient for complete separation of the carotene isomers. The writer finds that a second filtration of solutions of alpha and beta carotene through a column of calcium hydroxide, as described, increases the purity of the components (fig. 3). After the second elutriation, the respective alpha and beta carotene solutions are again concentrated to one-eighth their volume, and the carotenes crystallize immediately. After a recrystallization from petroleum ether (b.p. 35°–60°) and drying for six hours in a vacuum desiccator, the carotenes give the following constants:

	MELTING POINT	$a^{20}_{Cd.}$ (OPTICAL ROTATION)
Alpha carotene.....	187.5 ± 1° C.	390 ± 15
Beta carotene.....	187.5 ± 1° C.	0 ± 15

The yield is 19.5 mg. of alpha carotene and 113.0 mg. of beta carotene.

PURIFICATION OF LEAF XANTHOPHYLL

The method presented here was employed in the purification of twelve samples. Approximately 50 kg. of barley leaves (Wisconsin pedigree no. 38) are coarsely ground in a large food chopper and dried with the same precautions as carrot roots. The dried pulp is

again passed through the food chopper and then further pulverized by grinding in a burr mill. The total weight of the dried pulp is 4.0–4.4 kg.

The dried leaf powder is placed in a continuous extractor and the pigments completely extracted with acetone within 24 hours. After filtering the solution, it is concentrated to 1 liter under reduced pressure. Eighty grams of potassium hydroxide in 400 cc. methyl alcohol is added to the concentrated extract. The saponification is carried out as described in the purification of the carotenes.

After saponification, the solution is divided into two 700 cc. portions and each portion is transferred to a 4 liter glass separatory funnel containing 2 liters of distilled water. The carotenoids are removed from the saponified material by fractionation between carbon disulphide and the diluted acetone. It is necessary to pour the carbon disulphide through a small funnel in a manner which permits it to flow slowly down the side of the separatory funnel. After the separatory funnels are rotated gently, they are allowed to set for 30 minutes. The content of each separatory funnel is washed with three more 500 cc. portions of carbon disulphide. After the fourth portion is added, the separatory funnels are allowed to set for three hours before the carbon disulphide is drained off. The carbon disulphide portions are combined and washed with four 1 liter portions of distilled water. This removes the last traces of saponified material from the carotenoid solution. After the solution is dried with sodium sulphate, it is filtered through a 1.5 cm. layer of calcium carbonate. If the chlorophylls were not removed completely by saponification, they are removed in this filtration by preferential adsorption. The carotenoid solution is a deep clear red color.

After the solution is concentrated to 200 cc. under reduced pressure, 400 cc. of 89 per cent methyl alcohol is added and the solution is again concentrated to 200 cc. If all the carbon disulphide is not removed, another addition of 89 per cent methyl alcohol is made. The total volume of the solution is now increased to 2 liters by adding sufficient methyl alcohol to make the final concentration 89 per cent.

The carotenes are removed from the 89 per cent methyl alcohol solution by washing it with six 500 cc. portions of petroleum ether

(b.p. 60°–70°). The carotenes from this stage and on are purified as previously described. The xanthophyll solution is concentrated to 75 cc. The precipitated xanthophyll is a yellow color owing to the dilution of the solvent with water. The xanthophyll crystals melt at 168° C. The yield of this crude xanthophyll is 1.4 gm. In all the following recrystallizations, the same precautions to exclude oxygen are employed as described in the methods for the purification of the carotenes.

A 0.25 gm. sample of the crude xanthophyll is dissolved in 50 cc. of boiling absolute ethyl alcohol to which 100 cc. of petroleum ether (b.p. 30°–35°) is added. On cooling, the xanthophyll crystallizes immediately. After recovering the xanthophyll crystals by filtering this solution through a Berlin crucible, they are dissolved in 75 cc. of carbon disulphide. This solution is filtered through a 10 cm. layer of calcium carbonate which is made like the previously described 10 cm. calcium hydroxide layers. The adsorbed xanthophyll is washed with 25 cc. carbon disulphide. The resulting chromatogram consists of two or more zones. The top layer may vary from a white to a light red color depending on the kind and amount of impurities present. This layer is removed and discarded. The next zone, which comprises the major portion of the chromatogram, contains the adsorbed leaf xanthophyll.[2] Should other xanthophylls be present, the leaf xanthophyll will be in the lower zone (20). The color of the zone containing the leaf xanthophyll is a golden yellow. If carotenes are present the major portion will remain in the filtrate, and only a small amount will be adsorbed below the zone containing the leaf xanthophyll. After the leaf xanthophyll has been quickly elutriated with absolute ethyl alcohol, the xanthophyll solution is concentrated to 15 cc. under reduced pressure and set in the refrigerator. On cooling, crystallization of the xanthophyll occurs immediately. After recovering the xanthophyll crystals by filtration, they are washed with

[2] Since this paper was sent to press, it has been pointed out to the writer that the xanthophyll components observed by TSWETT should be exhaustively reinvestigated, especially in view of the fact that calcium carbonate is such a weak adsorbent. By the method presented here, the writer obtained the same absorption curve when leaf xanthophyll was isolated from either corn or barley leaves, which indicates that, if leaf xanthophyll consists of more than one component, the ratio between the components in this case remained fairly constant.

30 cc. of petroleum ether. After one hour's drying in a vacuum desiccator, the crystals melt at 185° C. The xanthophyll is dissolved in 22 cc. boiling absolute ethyl alcohol and cooled. To this solution is added an equal volume of petroleum ether, which causes the xanthophyll to crystallize. Following this crystallization, the xanthophyll is recrystallized from 17 and 15 cc. of methyl alcohol respectively. The final recrystallization is from 10 cc. of boiling carbon disulphide to which, after cooling, 30 cc. of petroleum ether (b.p. 30°–35°) is added. The dried xanthophyll crystals melt at 190° C. The yield is 95 mg.

The writer is indebted to several publications for the general method outlined here, the chief sources being the methods of WILLSTÄTTER and STOLL (**19**) and of SCHERTZ (**13**).

The lycopene employed in this study was furnished by MATLACK (**9**).

DISCUSSION OF CHROMATOGRAMS

The methods presented in this paper have shown that chromatographic analyses have extended applications in the purification of the carotenoids. The technique described, especially the packing of the adsorbent, was developed two years ago. The adsorbent then employed for the purification of the carotenes was "Fasertonerde" (Al_2O_3). Recently, KARRER (**5**) has reported that calcium hydroxide is a satisfactory adsorbent. The difficulties encountered in chromatographic analyses are (a) the use of unsuitable adsorbents, and (b) improper packing of the adsorption column.

It was found that ordinary commercial slacked lime does not possess sufficient affinity for either of the carotenes to give a satisfactory chromatographic separation. This same difficulty is encountered with many of the commercial hydroxides. The purified calcium hydroxide distributed by Central Scientific Company was employed exclusively for the chromatographic analyses presented in this paper.

As previously described, the adsorption column must be packed uniformly and firmly. This is achieved by firmly packing 1 cm. layers of the wet adsorbent. The edges of each layer are packed the firmest, otherwise the lines of demarcation between the zones are very irregular. For a layer 10 cm. thick, the firmer the calcium hydroxide is packed the more complete will be the separation of the

carotene isomers. The same technique is used when Fasertonerde is employed as the adsorbent.

When purifying alpha carotene by the method (**8**) employing fuller's earth as the adsorbent, it is necessary to start with a 3 gm. sample of carrot root carotene. The low yield by this method is due to contamination by oxidized carotenes. These oxidation products are formed by progressive oxidation, while the carotenes are on the surface of the adsorbent. The presence of these impurities prevents a large portion of alpha carotene from crystallizing in later purifications.

In carrying out chromatographic analyses, certain precautions must be observed: (1) The original sample must be fairly free from impurities, the purification being carried out in an inert atmosphere to minimize the formation of oxidation products. (2) The adsorbent as well as the carotenoid solution must be free from moisture. (3) The adsorbent should be finely powdered, enabling close packing of the particles and consequently the formation of a firm layer of the adsorbent. (4) The solution in the funnel containing the chromatogram must be protected from exposure to oxygen (fig. 2). If the formation of oxidation products is not prevented or removed as formed, the resulting carotenoid preparation after elutriation will fail to crystallize. (5) It is necessary to wash the chromatogram to ensure complete separation of the components. As washing proceeds, the different zones move down but retain their relative position to one another.

Discussion of yields

The yields of crude carotenes and xanthophyll by the methods presented here compare favorably with the highest reported in the literature. Escher found that carrot roots contained 0.0265 per cent as compared with 0.024 per cent by this method. The writer found that barley leaves contained 0.032 per cent (amount isolated) as compared with 0.012 and 0.04 per cent by the methods of Willstätter and Meig (**18**) and Jørgensen and Stiles (**3**) respectively. In the latter method the source of the carotenes was nettle leaves. All these yields are on a dry weight basis.

The highest yield was that obtained after the final purification

of alpha carotene, namely, 0.0195 gm. of alpha carotene from 0.31 gm. carrot root carotene, a yield of 6.22 per cent. SMITH (**14**), starting with a 3.05 gm. sample of carrot root carotene, succeeded in isolating 0.028 gm., a yield of 0.92 per cent. Hence the method presented here increased the yield of alpha carotene approximately sevenfold. It is also significant that SMITH was unable to salvage any beta carotene from the same sample, whereas by the writer's method the yield of beta carotene from the same sample was 35.9 per cent.[3]

Absorption spectra of purified carotenoids

The absorption spectra of alpha and beta carotene, leaf xanthophyll, and lycopene were studied by the spectro-photoelectric method developed by ZSCHEILE, HOGNESS, and YOUNG (**21**); and by MILLER (**12a**).[4] For this study 20 per cent anhydrous ether and 80 per cent absolute ethyl alcohol were used as the solvent. The solutions were changed after every fifth reading to avoid photodecomposition of the carotenoids. The values of the absorption coefficients as defined by Beer's law are plotted as the ordinates of the curves. From Beer's law:

$$I_x = I_0 \, 10^{-acx}$$

or

$$a = \log_{10} \frac{I_0}{\frac{I_x}{cx}}$$

I_0 = intensity of light transmitted by the solvent cell.
I_x = intensity of light transmitted by the solution cell.
x = thickness of the absorption cells (2.0 and 4.25 cm.).
c = concentration of the carotenoids in gm. per liter.

The nature of the absorption curves determined the intervals at which the final measurements were made. From these measurements the values of a were calculated and plotted as shown in figure 3.

[3] STRAIN has increased this yield. STRAIN H. H., Jour. Biol. Chem. **105**:523–535. 1934.

[4] Curves presented in figure 3 were obtained as already described except that the electrometer was replaced by a modified DuBridge circuit.

For different wave lengths, a was calculated from measurements made at different concentrations. When the values of a were plotted against wave lengths, it was found that the values of a for the different concentrations overlapped. This demonstrates that Beer's law is obeyed at the concentrations employed in this study, 0.0006 to 0.0012 gm. per liter. (The concentrations were limited by the thickness of the absorption cell employed.) The wave length readings are accurate to two Angstrom units. The experimentally determined values of a at maxima on the curves have an error of 1.3 per cent or less and ± 1.0 per cent for the rest of the respective absorption curves.

The magnitudes of the absorption coefficients at different wave lengths were employed as the final criteria of purity of the carotenoids. On pure samples, it was found that the values of a at certain wave lengths were approximately three times as sensitive and reliable as the criteria employing either melting points or optical rotation. The optical rotation method is reliable, but in this laboratory the spectro-photoelectric method was the most accurate. As the samples were purified, the resolution of the bands became more definite. The maxima of the bands increased and correspondingly the minima decreased, until the values of a presented graphically in figure 3 were obtained.

McNICHOLAS (**10**) measured the absorption coefficients of carotene (mixture of alpha and beta carotene) and leaf xanthophyll with a Koenig-Martin spectrophotometer. Those measurements are open to criticism because recent investigations have shown that neither of the carotenoid preparations employed was pure. In 1933, SMITH (**14**) photographed the absorption spectra of alpha and beta carotene on Eastman no. 40 plates with a Bausch and Lomb no. 2700 spectrometer and a no. 2750 photometer. By these methods it is difficult to detect the small bands that can readily be located by the spectro-photoelectric method. Slit widths employed were 0.015 mm. or less. Spectral range isolated was 7.5 A. units or less.

More nearly accurate measurements of the absorption spectra of alpha and beta carotene were reported by KUHN (**6**). He employed a spectro-photoelectric method for measuring the absorption coefficients of alpha and beta carotene in hexane and carbon disulphide.

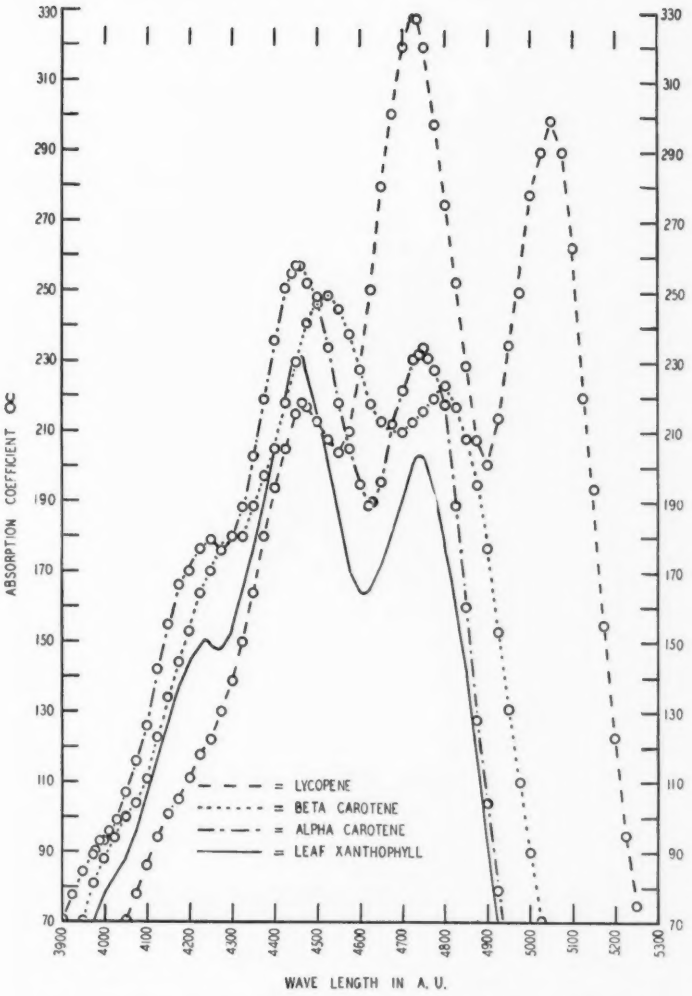


FIG. 3.—Absorption spectra of carotenoids in 20 per cent ether and 80 per cent absolute ethanol by volume.

The absorption spectrum of alpha carotene presented in figure 3 is similar to that obtained by KUHN. MCNICHOLAS (10) and SMITH (14) failed to find the band at 4050 A. units for alpha carotene, and the band at 4085 A. units for beta carotene.

Preparation of solvents

All solvents, as methyl alcohol, cyclohexane, chloroform, and petroleum ether fractions, were purified by careful distillations immediately before being used.

The acetone (c.p.) was redistilled. (b.p. 56.5° C.) The ammoniacal silver nitrate test for aldehydes was negative.

The ethyl alcohol (95 per cent) was allowed to stand over lime for four days. After removing the major portion of the lime by filtration, the ethyl alcohol was distilled.

The carbon disulphide had been used in previous extractions, but after distilling it boiled at 46.0°–46.4° C.

The diethyl ether (Baker's anhydrous) stood over sodium wire. It was distilled and stored in a brown bottle until used.

Tests of purity

1. MELTING POINTS.—This method is valid only when a substance is free from contamination of impurities possessing the same approximate melting points.

2. TESTS FOR PRESENCE OF OTHER ISOMERS.—The contamination of one isomer with others can be ascertained by the proper chromatographic analysis. It can also be determined by tests 3 and 4.

3. OPTICAL ROTATION METHOD.—This method is very satisfactory when the proper sources of monochromatic light are used with a polarimeter capable of determining rotations as small as 0.01°. The apparatus employed in this study permitted measurements to be made with an accuracy of 4 per cent error; hence this test was not employed as the final test of purity.

4. ABSORPTION SPECTRUM.—A critical spectroscopic examination of the absorption spectrum measured with an error of 1.3 per cent or less at maxima readily detects traces of impurities and contamination by other isomers. This method is accurate and precise.

Quantitative spectral analyses of binary mixtures of the purified carotenoids

A quantitative spectro-photoelectric method of analysis, in general, depends upon the precise determination of the fraction of light absorbed by a mixture of substances for at least as many different wave lengths as there are components for which analyses are to be made. The method may be exemplified by a brief consideration of the determination of the concentrations of alpha and beta carotenes in the same solution. In preparing for later analyses, it is first necessary to determine the absorption coefficients of pure components (alpha and beta carotene) in separate solutions (fig. 3) and over an extended wave length range. The purpose of this procedure is to permit the selection of the most suitable wave lengths of light to be employed in the analyses. These wave lengths are those for which there is an optimum difference between the absorption coefficients of the two components. Wave lengths 4862 and 4956 A. units were found to be satisfactory. Two or more wave lengths are employed for the purpose of check.

The average absorption coefficient of both carotenes in a mixture will be some value between the values of the coefficients of the pure components, and will be directly proportional to the relative amounts of the two components. The absorption coefficient of a mixture for a single wave length cannot be determined without a knowledge of the total amount of both carotenes, but if the absorption by the mixture is known for two wave lengths, then the absolute amounts of alpha and beta carotenes can be determined (two unknowns requiring two equations for solution). At 4500 A. units the absorption coefficients are equal for the carotenes. This wave length may be used to determine the total concentration, and one or more other wave lengths to determine the relative proportion of alpha and beta carotenes. The writer (11) has shown that for analytical purposes the percentage of composition of a component in a binary system can be calculated by the following equation:

$$a_T = (a_1 - a_2)\frac{C_1}{C_1 + C_2} + a_2 .$$

Tables I–III contain the results of analyses of several binary mixtures. The accuracy of the method may be seen by a comparison of the first and last rows. The last two columns (I and II) contain the determinations on different aliquots of the same unknown mixtures.

Analyses of beta carotene and leaf xanthophyll in grass tissues

A rapid and accurate quantitative method for extracting the carotenoids from green tissues has been described in detail by the writer (**12**). The weighed green samples are placed in a mortar (diameter 15 cm.) containing 25 cc. acetone and 25 gm. of quartz sand. The leaf tissue is macerated, and the acetone extract is decanted into a 250 cc. Erlenmeyer flask. The alternate maceration and extraction is repeated three times with 25 cc. portions of acetone and twice with 35 cc. portions of ether, the maceration and extraction requiring 12 to 15 minutes. Since all samples consist of succulent tissues, the sample is thoroughly disintegrated before the following extraction is made. The pulp and sand is collected in a washed cambray cloth bag which is placed in a Soxhlet extractor (size 18). About 100 cc. of ether is employed in transferring the pulp to the Soxhlet apparatus.

The extraction is completed by placing the Soxhlet extractor on a steam bath for 30 to 60 minutes. The quantity of carotenoids removed by the hot ether extraction is usually small. Analyses of several samples showed that this last extraction removed approximately 30 gamma from a 5.0 gm. sample. The chlorophylls and other esters are saponified by the addition of 20 cc. ethanol (95 per cent) saturated with potassium hydroxide to the combined ether and acetone extracts.

The extract is transferred to a 3 liter separatory flask containing 1.5 liter distilled water. The separatory funnel is whirled gently and allowed to set five minutes before the water is drained off into the second separatory funnel. If the saponification is not complete, an additional 10 cc. of ethanol saturated with potassium hydroxide is made. This second saponification may require 8 to 12 minutes. The content of the second separatory funnel is washed with 100 cc. ether. The ether washings are added to the first separatory funnel.

TABLE I

QUANTITATIVE SPECTRAL ANALYSES OF MIXTURES OF ALPHA AND BETA CAROTENE IN 20 PER CENT ETHER AND 80 PER CENT ETHANOL; COMPOSITION IN TERMS OF BETA CAROTENE

Wave length (A.U.)	Percentage known composition							Examples of analyses	
	5.0	10.0	25.0	50.0	75.0	90.0	95.0	I	II
4862	4.8	9.9	24.9	49.8	74.8	90.2	95.2	69.7	69.1
4956	5.4	10.1	25.1	50.0	74.8	90.3	94.8	67.5	68.2
Average percentage composition	5.1	10.0	25.0	49.9	74.8	90.2	95.0	68.6	68.7

TABLE II

QUANTITATIVE SPECTRAL ANALYSES OF MIXTURES OF BETA CAROTENE AND LYCOPENE IN 20 PER CENT ETHER AND 80 PER CENT ETHANOL; COMPOSITION IN TERMS OF LYCOPENE

Wave length (A.U.)	Percentage known composition							Examples of analyses	
	5.0	10.0	25.0	50.0	75.0	90.0	95.0	I	II
4956	5.2	9.8	24.9	49.7	74.6	89.7	94.6	30.5	29.6
5150	5.2	9.9	24.8	50.0	75.1	89.8	95.2	30.1	29.4
Average percentage composition	5.2	9.8	24.8	49.8	74.8	89.7	94.9	30.3	29.6

TABLE III

QUANTITATIVE SPECTRAL ANALYSES OF MIXTURES OF LYCOPENE AND LEAF XANTHOPHYLL IN 20 PER CENT ETHER AND 80 PER CENT ETHANOL; COMPOSITION IN TERMS OF LYCOPENE

Wave length (A.U.)	Percentage known composition							Examples of analyses	
	5.0	10.0	25.0	50.0	75.0	90.0	95.0	I	II
4722	5.4	10.2	25.6	50.6	75.7	90.0	95.4	34.5	34.1
5023	4.9	9.9	24.8	49.8	75.3	90.3	95.2	33.6	34.3
Average percentage composition	5.1	10.1	25.2	50.2	75.5	90.1	95.3	34.05	34.2

The ether solution in the first separatory funnel is washed four times with 500 cc. portions of distilled water. After each washing, the separatory funnel is stoppered and allowed to set two minutes, except the 5–8 minutes before the last washing is drained off.

TABLE IV

QUANTITATIVE SPECTRAL ANALYSES OF THE COMMON CAROTENOIDS

Plant	Number of sample	Percentage		
		Beta carotene	Leaf xanthophyll	Alpha carotene
Setaria stramineofructa	101	0.00753	0.03110	0.0000
	102	0.00740	0.03060	0.0000
Average		0.00746	0.03080	0.0000
Setaria italica	103	0.00840	0.02210	0.0000
	104	0.00820	0.02200	0.0000
Average		0.00830	0.02205	0.0000
Hordeum sativum (Wis. Ped. no. 38)	105	0.00327	0.00840	0.0000
	106	0.00308	0.00840	0.0000
Average		0.00317	0.00840	0.0000
Dactylis glomerata	107	0.00408	0.01482	0.0000
	108	0.00443	0.01490	0.0000
Average		0.00425	0.01486	0.0000
Bromus inermis	109	0.00537	0.02360	0.0000
	110	0.00490	0.02410	0.0000
Average		0.00513	0.02380	0.0000
Zea mays (var. Country gentleman)	111	0.00454	0.02196	0.0000
	112	0.00470	0.02130	0.0000
Average		0.00462	0.02163	0.0000
Zea mays (var. Golden bantam)	113	0.00663	0.02185	0.0000
	114	0.00683	0.02250	0.0000
Average		0.00373	0.02217	0.0000
Festuca elatior	119	0.00604	0.02176	0.0000
	120	0.00625	0.02295	0.0000
Average		0.00615	0.02235	0.0000

The ether solution (150–200) is carefully transferred to a 500 cc. balloon flask, 25 cc. of ether being used for washing the separatory funnel. The ether solution in vacuo is evaporated to 50–60 cc. The carotenoid solution is transferred to a 100 cc. graduate and meas-

ured. The ether solution is poured into a 500 cc. volumetric flask. The balloon flask and graduate are washed with sufficient ether to make the total volume 100 cc. The solution is made up to volume by the addition of 400 cc. of absolute ethanol. It is usually necessary to make further dilutions before analyses are made. Quantitative spectral analyses of the carotenoids from eight grasses are summarized in table IV. Thus, by this spectro-photoelectric method it is possible to analyze plant extracts and to determine the total concentration of each component.

Summary

1. Simple improved methods for the purification of three of the common carotenoids have been developed. The methods of TSWETT and ESCHER have been combined for the purification of the carotene isomers. The methods are described in detail.

2. A critical discussion of chromatographic analysis has been made. The necessary precautions are listed.

3. The quantitative absorption spectra of alpha and beta carotene, leaf xanthophyll, and lycopene were measured with an error of 2.0 per cent or less between 3950 and 5500 A. units. These spectra were measured by a spectro-photoelectric method which is accurate and precise.

4. Spectroscopic data show that certain tests of purity which have been considered accurate are only approximate. It is concluded that a critical examination of the absorption spectrum is the most reliable.

5. The absorption spectra of the carotenoids are employed as the basis for quantitative spectral analytical methods which have been applied to binary mixtures.

6. The preparative procedure includes and employs the precaution of excluding oxygen in the important steps of purification.

The writer wishes to express his appreciation to those who have given him the opportunity to make this investigation: to Prof. T. R. HOGNESS for his numerous suggestions regarding absorption spectra measurements and quantitative spectral analyses; to the Department of Botany, University of Chicago, for plants needed in the

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TOXICITY OF PHENOLIC COMPOUNDS TO CERTAIN ONION BULB PARASITES[1]

J. C. WALKER AND KARL PAUL LINK

I. Introduction

Resistance of colored varieties of onion to *Colletotrichum circinans* (Berk.) Vogl. is associated with the presence of water-soluble toxic substances in the dry outer scales of the bulbs (**22, 23**). Biochemical studies of extracts from colored scales have shown that the latter contain two phenolic substances, namely protocatechuic acid (3, 4 dihydroxy benzoic acid) (**2, 13, 14**) and catechol (1, 2 dihydroxy benzol) (**15**). These compounds, in concentrations of 1–800 and 1–1600 respectively, are strongly toxic to *C. circinans*, and are considered responsible, in part at least, for the resistance of colored bulbs.

The importance of phenolic compounds in plant tissue in warding off or restricting the attack of pathogenic organisms has been suggested by a number of workers. The work of COOK and TAUBENHAUS (**5, 6**), and COOK and WILSON (**7**) indicated that tannin compounds were toxic in certain concentrations to a great number of fungi. GRAVES (**10**) showed a distinct correlation of resistance to *Endothia parasitica* (Mur.) A. & A. with high tannin content of chestnut roots, while susceptibility of the bark of the stem was correlated with low tannin content. MARAÑON (**16**) found those strains of *Oenothera* which were resistant to *Erysiphe polygoni* DC. to be higher in tannin content than were susceptible strains.

Tannins and related compounds contain certain of the simpler phenolic compounds such as gallic acid, catechol, and protocatechuic acid. NEWTON and ANDERSON (**17**) offered the hypothesis that resistance in wheat to *Puccinia graminis* Pers. was due to phenolic compounds set free in the host cell by the action of fungal enzymes upon more complex substances. It was also suggested by them that

[1] This investigation was conducted by the Department of Plant Pathology and the Biochemical Research Laboratory, Department of Agricultural Chemistry, University of Wisconsin, in cooperation with the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture. It was supported in part by a grant from the University Research Fund.

susceptibility might be attributed to the presence of enzyme inactivators which prevented the fungus from setting free the phenolic compounds. Such a theory does not apply to the case of resistance in the onion. The phenolic compounds, catechol and protocatechuic acid, occur in the non-living tissue of the outer scales and diffuse into the soil moisture exterior to the colored bulb. If the fungus is present on the exterior its growth is inhibited and infection prevented. When this barrier of dead outer scales is removed, however, and the inoculum placed directly on the fleshy scales of colored bulbs, penetration and further invasion is exactly similar to that in bulbs of susceptible varieties. As pointed out in an earlier paper (**22**), the epidermal cells of the fleshy scales of colored varieties contain phenolic pigment compounds in the cell sap. The pigment is destroyed, however, as soon as penetration of the host cuticle is accomplished by the parasite and before the cell lumen containing the phenolic compounds is invaded by the fungus hyphae.

There is little experimental work to be found wherein the simpler phenolic compounds have been critically compared as to their effects upon fungous growth. NEWTON, LEHMANN, and CLARKE (**18**) reported studies with salicylic acid, catechol, and vanillin as they affected growth of *Helminthosporium sativum* P. K. & B. in Knop's nutrient solution. Salicylic acid was the most toxic and vanillin the least toxic. When added in very small amounts, however, all stimulated growth.

The present paper reports a study of the influence of twenty-one phenolic compounds upon four fungi. In addition to *C. circinans*, there were included two onion-bulb pathogens, *Botrytis allii* Munn and *Aspergillus niger* Van T. *Gibberella saubinetii* (Mont.) Sacc., a fungus pathogenic on wheat and corn but not known to attack the onion, was the fourth organism used. Colored onion varieties are not only highly resistant to *C. circinans* but they are also usually relatively free from infection by *Botrytis allii*. *Aspergillus niger*, however, attacks both colored and white bulbs (**23**).

II. Methods

All the compounds studied were purified by appropriate methods until their physical constants corresponded to the accepted values

reported in the literature (1, 12, 20).[2] They were added in various amounts to modified Czapek's nutrient solution.[3] Fifty cc. aliquots of the medium were placed in 250 cc. flasks and sterilized in the autoclave. The inoculum for each flask consisted of 1 cc. of a suspension of the spores in sterile distilled water. The concentration of spores of a given organism was thus the same throughout any given dilution series. It was not the same for every experiment, however, and this accounts in part for the variation in the weights of the fungus between controls of different experiments with the same fungus. The cultures were incubated at room temperature, which varied from 22° to 24° C. in some of the series and from 26° to 28° C. in others. Each series was run in triplicate.

When the mycelial mats had reached the fruiting stage they were removed by filtering the cultures through weighed filter papers on a Büchner funnel. This was usually on the fourth day after inoculation with *Aspergillus niger* and on the sixth day with the other organisms. The paper and the fungus mat were dried in a vacuum oven at 80° C. under 12 mm. pressure and weighed.

With the data thus secured it was possible to compare the compounds with one another on the basis of the lowest concentration required to be incorporated in Czapek's solution for complete inhibition of germination and growth. This concentration will be referred to as the inhibitive concentration. It was also possible to compare the compounds as to the extent to which they retarded or stimulated growth of the fungi in Czapek's solution when present in the latter at various concentrations lower than that at which complete inhibition occurred.

III. Results

The compounds studied will be considered in three groups. In the first group are the various phenols and substituted phenols; in the second are benzoic acid, various phenolic acids, and oxalic acid;[4] and

[2] Acknowledgment is made to Dr. SAM MORELL, Research Assistant in Biochemistry, for assistance in purification of the substances used and for the synthesis of the derivatives of protocatechuic acid.

[3] Magnesium sulphate, 0.5 gm.; monobasic potassium phosphate, 1.0 gm.; potassium chloride, 0.5 gm.; ferrous sulphate, 0.01 gm.; asparagin, 1.0 gm.; d-glucose, 30 gm.; water, 1000 cc.

[4] Oxalic acid was included because of its wide occurrence in plant tissues in the free state or as an oxalate, and also because of its toxic properties.

in the third group are a number of derivatives of protocatechuic acid. All four organisms were tested against most of the compounds. The data secured with *Colletotrichum circinans* are presented first, followed by the comparative results with the other organisms.

TABLE I

GROWTH OF COLLETOTRICHUM CIRCINANS ON CZAPEK'S SOLUTION CONTAINING PHENOLS IN VARIOUS CONCENTRATIONS

PHENOL	DRY WEIGHT (MG.) OF FUNGUS AT VARIOUS CONCENTRATIONS											
	CONTROL	1–100	1–200	1–400	1–800	1–1600	1–3200	1–6400	1–12,800	1–25,600	1–51,200	1–102,400
$C_6H_5 \cdot OH$ Phenol	92	0	0	0	0	0	14	36	.....	.....	.....	.....
	108	.....	.....	.....	.....	0	25	40	60	92	80	.....
	93	.....	.....	.....	0	0	30	55	60	51	77	73
$C_6H_4 \cdot (OH)_2$ 1:2 Catechol	89	...	0	0	0	0	0	10	25	69	.....	.....
	176	.....	.....	.....	0	0	23	62	75	82	95	115
	135	.....	.....	.....	0	0	10	29	45	65	80	101
$C_6H_4 \cdot (OH)_2$ 1:3 Resorcinol	118	...	0	0	44	75	93	103	122	119	140	.....
	35	.....	.....	0	14	17	24	29	30	35	.....	.....
$C_6H_4 \cdot (OH)_2$ 1:4 Hydroquinone	92	...	0	0	0	0	10	19	39	56	78	.....
	38	.....	.....	.....	0	11	15	28	45	45	56	.....
$C_6H_3 \cdot (OH)_3$ 1:3:5 Phloroglucinol	36	6	13	15	21	25	23	26	.....	.....	.....	.....
	35	...	10	17	18	29	36	38	.....	.....	.....	.....
	93	...	36	66	88	115	111	115	142	151	.....	.....
$C_6H_3 \cdot (OH)_3$ 1:2:3 Pyrogallol	51	.....	.....	.....	0	0	0	0	0	10	28	34
	108	.....	.....	.....	.....	.....	.....	.....	0	48	95	125
$C_6H_4 \cdot OH \cdot OCH_3$ 1:2 Guaiacol	135	.....	.....	.....	0	0	111	126	145	157	143	141
	176	.....	.....	.....	0	0	127	165	185	175	217	200
$C_6H_4 \cdot (OCH_3)_2$ 1:2 Veratrol	135	.....	.....	.....	0	0	136	138	133	144	135	141
	176	.....	.....	.....	0	0	155	165	155	183	161	171

TOXICITY OF PHENOLS TO COLLETOTRICHUM CIRCINANS.—The following phenols and substituted phenols were used: phenol; the three dihydroxy phenols, catechol, resorcinol, and hydroquinone; two trihydroxy phenols, phloroglucinol and pyrogallol; and guaiacol and veratrol, both of which are methyl derivatives of catechol. The results of growth studies in Czapek's solution are given in table I.

When these compounds are compared there appears to be no relation between molecular weight and toxicity. The position of the

hydroxyl groups in relation to one another, however, does have a distinct effect upon toxicity to *C. circinans*. The three dihydroxy isomers illustrate this fact clearly. Catechol, the ortho compound, was about equal to phenol. The inhibitive concentration was 1–1600. The weight of the fungus increased gradually with dilution of catechol but did not equal that of the control even at 1–102,400. Resorcinol, the meta compound, was much less toxic. The inhibitive concentration was 1–400 while normal growth occurred at 1–12,800 in one experiment and at 1–25,600 in the other. Hydroquinone, the para compound, was nearly as toxic as catechol and phenol.

A comparison of the two trihydroxy phenols shows a still wider range of toxicity between isomers. In phloroglucinol the hydroxyl groups are in the meta position to one another as in resorcinol, while in pyrogallol they are in the ortho position, as in catechol. Phloroglucinol was still less toxic than resorcinol. The inhibitive concentration was not determined, but growth occurred at 1–200 in three trials and at 1–100 in the one trial made at the latter concentration. Growth equal to the control occurred at 1–1600 in one trial and at 1–3200 in another. There was some stimulation at greater dilutions in one experiment. On the other hand, pyrogallol, with an inhibitive concentraton of 1–12,800, was the most toxic of all phenols tested.

It is clear that, starting with phenol, the addition of one hydroxyl group in the meta position (resorcinol) reduces toxicity and changes the inhibitive concentration from 1–1600 to 1–400, while the addition of another hydroxyl group, in the meta position to the second (phloroglucinol), changes the inhibitive concentration to more than 1–100. On the contrary, when one hydroxyl group is added to phenol in the ortho position (catechol), toxicity is not changed materially, but when another hydroxyl is added in the ortho position to the second (pyrogallol), toxicity is increased abruptly and the inhibitive concentration is changed to 1–12,800. COOPER and MASON (**8**), in their studies with *Bacillus coli* and *B. fluorescens non-liquefaciens*, found the toxicity of resorcinol and phloroglucinol less than that of phenol, and CAIUS, NAIDU, and SHAMSHER (**4**) reported the same for *Bacillus pestis*.

Guaiacol and veratrol are methyl derivatives of catechol. In the former, the methyl group is substituted for hydrogen in one of the

hydroxyl groups; in the latter, substitution occurs in both hydroxyl groups. In neither case was the inhibitive concentration changed from that of catechol. Retardation in growth, however, was more readily diminished with increase in dilution in the case of methyl derivatives than in that of catechol. At the 1–102,400 concentration of the latter, weight of the fungus was still less than that of the control. Guaiacol, however, did not retard growth at 1–12,800 and seemed to stimulate it at higher dilutions. Veratrol did not reduce growth at 1–3200 in one experiment and did so only slightly in another, but there was no stimulation at greater dilutions.

Toxicity of phenolic acids to Colletotrichum circinans.—The following phenolic acids were studied: salicylic, meta-hydroxy benzoic, para-hydroxy benzoic, protocatechuic, gallic, and anesic. Benzoic and oxalic acids were added to the list for comparative purposes. The results of growth studies in Czapek's solution to which the acids were added in various concentrations are given in table II.

Benzoic acid was decidedly more toxic than any of its phenolic derivatives which were tested. The three isomeric monohydroxy benzoic acids differed from one another in toxicity and the variation resembled in a general way that which existed between the corresponding dihydroxy phenols. Thus salicylic acid, in which the hydroxyl and carboxyl groups are in the ortho position to one another, was the most toxic, with an inhibitive concentration of 1–6400. Para-hydroxy benzoic acid, in which they are in the para position to one another, was next in the descending order of toxicity, with an inhibitive concentration of 1–1600. Growth occurred in a 1–1600 concentration of the meta form, meta-hydroxy benzoic acid, and the inhibitive concentration, although not determined, was at 1–800 or greater. The monohydroxy acids therefore assume the same relative order of toxicity as the corresponding dihydroxy phenols: catechol, hydroquinone, and resorcinol. Salicylic acid reduced growth, as compared with the control, up to rather high dilutions (1–204,800); on the other hand, retardation of growth by the meta- and para-hydroxy acids was diminished more readily by dilution, for their effects were negligible at 1–6400 concentrations. This was also usually true for the meta- and para-hydroxy phenols, resorcinol and hydroquinone, as compared with the ortho form, catechol.

Only one dihydroxy benzoic acid (protocatechuic) and one trihydroxy acid (gallic) were available for study. It is thus not possible to compare either of these with its respective isomers. Protocatechuic acid was about equal in toxicity to meta-hydroxy benzoic acid, from which it differed only in the presence of a second hydroxyl group in

TABLE II

GROWTH OF COLLETOTRICHUM CIRCINANS ON CZAPEK'S SOLUTION CONTAINING PHENOLIC ACIDS, BENZOIC ACID, AND OXALIC ACID IN VARIOUS CONCENTRATIONS

Acid	Dry weight (mg.) of fungus at various concentrations										
	Control	1–400	1–800	1–1600	1–3200	1–6400	1–12,800	1–25,600	1–51,200	1–102,400	1–204,800
$C_6H_5 \cdot COOH$ Benzoic	48	...	...	...	0	0	0	0	58	79	77
	108	...	0	0	0	0	0	0	30	62	62
$C_6H_4 \cdot COOH \cdot OH$ 1:2 Salicylic	81	...	...	...	0	0	53	56	67	.....	63
	108	...	...	...	...	0	20	32	38	70	90
$C_6H_4 \cdot COOH \cdot OH$ 1:3 Meta-hydroxy benzoic	42	...	...	...	13	39	30	32	46	.....	.....
	107	...	...	9	80	136	146	149	191	.....	.....
$C_6H_4 \cdot COOH \cdot OH$ 1:4 Para-hydroxy benzoic	48	...	0	0	28	45	79	64	43	46	.....
	38	...	0	0	15	28	35	55	50	.....	.....
$C_6H_3 \cdot COOH \cdot (OH)_2$ 1:3:4 Protocatechuic	36	0	0	0	23	25	29	34	.....	.....	.....
	81	0	0	25	57	73	66	70	.....	.....	.....
	31	...	0	6	18	27	28	26	.....	.....	.....
$C_6H_2 \cdot COOH \cdot (OH)_3$ 1:3:4:5 Gallic	78	0	15	25	20	35	42	54	.....	.....	.....
	35	0	11	16	25	20	35	48	.....	.....	.....
$C_6H_4 \cdot COOH \cdot OCH_3$ 1:4 Anesic	108	...	...	...	...	...	...	8	60	25	100
	42	...	...	...	...	...	...	...	4	13	14
$(COOH)_2 \cdot 2H_2O$ Oxalic	64	...	...	9	40	45	49	51	60	48	52
	38	...	0	0	0	15	43	55	55	.....	.....

ortho position to the first hydroxyl. Gallic acid may be looked upon as protocatechuic acid in which a third hydroxyl group is added in the ortho position. This addition resulted in lower toxicity.

Thus we may point out the following series in which the addition of hydroxyl groups results in reduced toxicity as measured by the respective inhibitive concentrations: benzoic acid, 1–25,600; meta-hydroxy benzoic acid in which one hydroxyl group is added in the

1:3 position, 1–800 or higher; protocatechuic acid in which two hydroxyl groups are added in the 1:3:4 positions, 1–800; and gallic acid in which three hydroxyl groups are added in the 1:3:4:5 positions, 1–400.

Anesic acid, the methyl derivative of para-hydroxy benzoic acid, is distinctly more toxic than the latter. Oxalic acid is less toxic than benzoic and more toxic than the weakest phenolic acid tested (gallic).

TOXICITY OF PROTOCATECHUIC ACID DERIVATIVES TO C. CIRCINANS.—In the tests of phenols and phenolic acids just discussed certain methyl derivatives were included. The two derivatives of catechol (guaiacol and veratrol) prevented growth at the same concentration as catechol. At the higher dilutions, however, the toxicity of the methyl derivatives was more rapidly reduced and in the case of guaiacol there was some evidence of stimulation. On the other hand, anesic acid was much more toxic than para-hydroxy benzoic acid from which it is derived.

In view of our particular interest in protocatechuic acid, several of its derivatives were tested. These are listed and the data secured with each are given in table III. The acid prevented growth at 1–800. The methyl and ethyl esters were more toxic, both having inhibitive concentrations of 1–1600. The methyl derivatives (vanillic acid and veratric acid) were inhibitive also at 1–1600, but, like the methyl derivatives of catechol, the toxicity was reduced rapidly by dilution. Thus they were both decidedly stimulative at 1–12,800, while protocatechuic acid and the esters usually retarded growth perceptibly at 1–25,600. The diacetyl derivative was inhibitive at 1–3200 and retarded growth at dilutions as high as 1–51,200. Protocatechuic aldehyde inhibited the fungus at the same concentration as the acid but when diluted to 1–12,800 it stimulated growth. The methyl derivative of the aldehyde, vanillin, was slightly more toxic, but in one experiment stimulated growth at 1–6400 and lower concentrations.

SUMMARY OF RESULTS WITH COLLETOTRICHUM CIRCINANS.—It is shown in the foregoing experiments that phenolic compounds vary widely in their toxicity to *C. circinans*. Variation is as great between certain isomeric compounds as between more widely separated groups.

The position of the hydroxyl groups in relation to each other has an important bearing on the inhibitive concentration. Thus in the dihydroxy phenol and the monohydroxy phenolic acid series the

TABLE III

GROWTH OF COLLETOTRICHUM CIRCINANS ON CZAPEK'S SOLUTION CONTAINING PROTOCATECHUIC ACID AND CERTAIN OF ITS DERIVATIVES IN VARIOUS CONCENTRATIONS

COMPOUND	DRY WEIGHT (MG.) OF FUNGUS AT VARIOUS CONCENTRATIONS								
	CONTROL	1–800	1–1600	1–3200	1–6400	1–12,800	1–25,600	1–51,200	1–204,800
$C_6H_3 \cdot COOH \cdot (OH)_2$ 1:3:4 Protocatechuic acid	36	0	0	23	25	29	34	.....	.....
	81	0	25	57	73	66	70	.....	.....
	31	0	6	18	27	28	26	.....	.....
$C_6H_3 \cdot COOCH_3 \cdot (OH)_2$ 1:3:4 Methyl ester of protocatechuic acid	36	0	0	12	30	25	27	.....	.....
	31	0	0	13	28	31	30	.....	.....
$C_6H_3 \cdot COOC_2H_5 \cdot (OH)_2$ 1:3:4 Ethyl ester of protocatechuic acid	36	0	0	22	22	24	25	.....	.....
	31	0	0	28	23	27	26	.....	.....
$C_6H_3 \cdot COOH \cdot (OC{-}CH_3)_2$ 1:3:4 Diacetyl protocatechuic acid	36	0	0	0	19	24	26	26	31
	31	....	....	0	15	20	20	24	31
$C_6H_3 \cdot COOH \cdot OH \cdot OCH_3$ 1:3:4 Vanillic acid	107	....	0	64	135	189	174	176	.....
	42	....	....	0	3	34	48	41	.....
$C_6H_3 \cdot COOH \cdot (OCH_3)_2$ 1:3:4 Veratric acid	176	0	0	17	26	196	200	180	175
	135	0	0	19	33	180	193	178	133
$C_6H_3 \cdot CHO \cdot OCH_3 \cdot OH$ 1:3:4 Vanillin	42	....	0	0	21	21	21	.....	.....
	107	....	0	40	128	131	154	204	.....
$C_6H_3 \cdot CHO \cdot (OH)_2$ 1:3:4 Protocatechuic aldehyde	93	0	3	55	83	126	135	.....	.....
	42	0	0	6	40	55	55	.....	.....

ortho compounds exhibit the greatest toxicity while the meta compounds are the least toxic. In the trihydroxy phenols, phloroglucinol with a 1:3:5 arrangement of hydroxyl groups is very low in toxicity, while its isomer, pyrogallol, with a 1:2:3 arrangement is the most toxic of the phenols tested. Protocatechuic acid is about equal to

meta-hydroxy benzoic acid while the trihydroxy acid (gallic) is less toxic.

Guaiacol and veratrol, the methyl derivatives of catechol, have the same inhibitive concentration as the latter. Vanillic and veratric

TABLE IV

GROWTH OF FOUR FUNGI ON CZAPEK'S SOLUTION CONTAINING PHENOLS IN VARIOUS CONCENTRATIONS

PHENOL	ORGANISM*	DRY WEIGHT (MG.) OF FUNGI AT VARIOUS CONCENTRATIONS										
		CONTROL	1–100	1–200	1–400	1–800	1–1600	1–3200	1–6400	1–12,800	1–25,600	1–51,200
$C_6H_5 \cdot OH$ Phenol	C.c.	92	0	0	0	0	0	14	36	.....	.....	.....
	G.s.	123	0	0	0	0	0	32	71	.....	.....	.....
	B.a.	273	0	0	0	0	40	151	209	216	284	293
	A.n.	261	0	0	0	0	53	243	262	.....	.....	.....
$C_6H_4 \cdot (OH)_2$ 1:2 Catechol	C.c.	89	0	0	0	0	0	0	10	25	69	.....
	G.s.	63	0	0	0	0	0	16	30	36	45	.....
	B.a.	227	0	0	0	0	54	140	186	170	206	.....
	A.n.	162	0	0	47	104	155	142	145	159	145	.....
$C_6H_4 \cdot (OH)_2$ 1:3 Resorcinol	C.c.	118	....	0	0	44	75	93	103	122	119	140
	G.s.	150	....	0	49	77	104	117	125	157	149	163
	B.a.	274	....	45	142	190	175	250	243	284	300	264
	A.n.	218	....	0	164	154	169	155	219	215	211	220
$C_6H_4 \cdot (OH)_2$ 1:4 Hydroquinone	C.c.	92	....	0	0	0	0	10	19	39	56	78
	G.s.	92	....	0	0	13	37	31	45	42	46	44
	B.a.	120	....	0	0	23	99	116	129	178	175	152
	A.n.	235	....	161	164	172	190	215	215	213	178	203
$C_6H_3 \cdot (OH)_3$ 1:3:5 Phloroglucinol	C.c.	36	6	13	15	21	25	23	26	.....	.....	.....
	G.s.	109	115	133	127	143	134	148	139	.....	.....	.....
	B.a.	78	84	67	59	49	71	91	84	.....	.....	.....
	A.n.	266	224	238	230	221	220	219	245	.....	.....	.....
$C_6H_3 \cdot (OH)_3$ 1:2:3 Pyrogallol	C.c.	51	.....	.....	.....	0	0	0	0	0	10	28
	G.s.	107	.....	.....	.....	0	0	0	13	15	16	88
	B.a.	88	.....	.....	.....	0	0	0	4	6	8	26
	A.n.	91	31	44	49	84	104	121	89	90	88	.....

* Abbreviations used in this table and table V are: C.c. = *Colletotrichum circinans;* G.s. = *Gibberella saubinetii;* B.a. = *Botrytis allii;* A.n. = *Aspergillus niger.*

acids, the methyl derivatives of protocatechuic acid, have a lower inhibitive concentration than the latter. When used in greater dilutions, however, the four methyl derivatives do not remain as toxic as the respective compounds from which they are derived. In fact they

TABLE V

GROWTH OF FOUR FUNGI ON CZAPEK'S SOLUTION CONTAINING OXALIC, BENZOIC, AND CERTAIN PHENOLIC ACIDS IN VARIOUS CONCENTRATIONS

ACID	ORGANISM*	DRY WEIGHT (MG.) OF FUNGI AT VARIOUS CONCENTRATIONS											
		CONTROL	1–200	1–400	1–800	1–1600	1–3200	1–6400	1–12,800	1–25,600	1–51,200	1–102,400	1–204,800	1–409,600
$C_6H_5 \cdot COOH$ Benzoic	C.c.	48	.....	.....	.....	.....	0	0	0	0	58	79	77	64
	G.s.	120	.....	.....	.....	.....	0	0	0	0	83	96	114	127
	B.a.	125	.....	.....	.....	.....	0	0	0	10	76	99	76	76
	A.n.	114	.....	.....	0	0	0	30	83	108	134	99	108	93
$C_6H_4 \cdot COOH \cdot OH$ 1:2 Salicylic	C.c.	81	.....	.....	.....	.....	0	0	53	56	67	.....	63	66
	G.s.	105	.....	.....	.....	.....	0	0	0	12	69	114	120	90
	B.a.	188	.....	.....	.....	.....	0	0	49	139	190	205	270	194
	A.n.	295	.....	.....	.....	.....	0	153	163	225	229	210	225	252
$C_6H_4 \cdot COOH \cdot OH$ 1:4 Para-hydroxy benzoic	C.c.	48	.....	.....	0	0	28	45	79	64	43	46	.....	.....
	G.s.	110	.....	.....	0	33	41	38	91	76	68	44	.....	.....
	B.a.	53	.....	.....	0	4	38	41	50	40	48	41	.....	.....
	A.n.	167	43	41	69	69	109	125	132	107	88	81	.....	.....
$C_6H_3 \cdot COOH(OH)_2$ 1:3:4 Protocatechuic	C.c.	81	0	0	0	25	57	73	66	70	.....	.....	.....	.....
	G.s.	63	0	10	69	86	52	61	72	67	.....	.....	.....	.....
	B.a.	176	78	131	129	116	122	153	162	171	.....	.....	.....	.....
	A.n.	209	216	214	217	234	138	223	204	191	.....	.....	.....	.....

* For explanation of abbreviations used see footnote to table IV.

$C_6H_2 \cdot COOH \cdot (OH)_3$ 1:3:4:5 Gallic	C.c.	78	0	0	15	25	20	35	42	54	.....	.....	.....	.....	.....	.....	.....
	G.s.	76	0	23	25	69	92	77	88	78	.....	.....	.....	.....	.....	.....	.....
	B.a.	129	13	16	16	24	32	44	67	91	.....	.....	.....	.....	.....	.....	.....
	A.n.	184	204	209	240	243	253	278	313	317	.....	.....	.....	.....	.....	.....	.....
$C_6H_4 \cdot COOH \cdot OCH_3$ 1:4 Anesic	C.c.	42	.....	.....	.....	.....	.....	.....	.....	0	4	13	14	19			
	G.s.	85	.....	.....	.....	.....	.....	.....	.....	0	71	73	78	75			
	B.a.	188	.....	.....	.....	.....	.....	.....	.....	0	68	80	74	78			
	A.n.	77	.....	.....	.....	.....	.....	.....	.....	53	58	66	43	44			
$(COOH)_2$ Oxalic	C.c.	64	.....	.....	.....	9	40	45	49	51	60	48	52	.....			
	G s.	82	.....	.....	.....	20	27	38	43	47	80	69	89	.....			
	B.a.	146	.....	.....	.....	113	125	158	144	161	199	192	195	.....			
	A.n.	207	.....	.....	.....	179	209	203	212	229	226	242	253	.....			

stimulate growth of the fungus in excess of the control at certain concentrations.

Other compounds in which there is a relatively narrow range between the inhibitive concentration and that at which growth is no longer retarded and sometimes stimulated are: meta-dihydroxy phenol (resorcinol), meta-hydroxy benzoic acid, protocatechuic aldehyde, and its methyl derivative vanillin.

TOXICITY OF PHENOLIC COMPOUNDS TO OTHER FUNGI.—As indicated earlier in the paper, the study of certain of the compounds was extended to *Gibberella saubinetii*, *Botrytis allii*, and *Aspergillus niger*. The results with phenols are given in table IV. In general the four fungi assume the following increasing order of tolerance to each compound: *C. circinans*, *G. saubinetii*, *B. allii*, and *A. niger*. One exception is resorcinol of which *B. allii* is more tolerant than is *A. niger*. The range of tolerance within this group of fungi was not the same for each compound. For instance, with pyrogallol the inhibitive concentration for *C. circinans* was 1–12,800, while for *A. niger* it was above 1–100. By contrast, the inhibitive concentration of phenol for *C. circinans* was 1–1600 and for *A. niger*, 1–800. In the case of resorcinol, where there was an indication of slight stimulation of *C. circinans* at certain dilutions, the same was true for *G. saubinetii* and *B. allii* but not for *A. niger*. *B. allii* was also stimulated at certain dilutions of phenol and hydroquinone while *A. niger* was stimulated by pyrogallol.

The results with certain of the acids are given in table V. The same relative order of toxicity of the acid compounds observed earlier with *C. circinans* held for the other three fungi. *C. circinans* again was invariably the least tolerant and *A. niger* was in all cases the most tolerant of the four organisms. Protocatechuic acid and gallic acid did not prevent nor retard growth of *A. niger* in concentrations as high as 1–200. In fact this organism was stimulated to greater growth than that of the control in all concentrations of gallic acid used. The tolerance of this organism to these phenolic compounds is not surprising in view of the fact that BUTKEWITSCH (3) found it could break down quinic acid to form protocatechuic acid and catechol.

IV. Discussion

A review of the literature reveals the fact that, in spite of the work of EHRLICH and others, our knowledge of the relations of the structure of phenolic bodies to their antiseptic and toxic properties is still very incomplete (**9, 19**). It has been shown by various investigators that the germicidal activity in homologous series of alcohols and phenols increases with molecular weight. JOHNSON and LANE (**11**) showed that the antiseptic strength of resorcinol increased as alkyl radicals of greater weight were introduced directly into the benzene nucleus. TILLEY and SCHAFFER (**21**) found increasing germicidal value of normal alcohols with each additional methyl group in the chain. When isomeric alcohols were compared, however, a regular decrease in germicidal value from primary to secondary to tertiary normal alcohols was noted. The structure of the molecule and the molecular weight both influenced the degree of toxicity.

The concentrations necessary to prevent growth, as well as the growth rates at concentrations lower than that required for inhibition, were considered in the present study. In the compounds tested the molecular weight appeared to have little influence in determining relative toxicity. In the phenols the position of the hydroxyl groups in relation to one another affects toxicity. When the hydroxyls are arranged in meta position to one another there is a descending order of toxicity with increase in molecular weight, namely, phenol, resorcinol, phloroglucinol. When the hydroxyls are arranged in ortho position there is an ascending order of toxicity with increase in molecular weight, namely, phenol, catechol, pyrogallol. In the three isomeric monohydroxy benzoic acids the relation of the hydroxyl to the carboxyl group is the important structural feature of the molecules. Benzoic, meta-hydroxy, protocatechuic, and gallic acids make up a descending order of toxicity to *C. circinans* with increase in molecular weight.

The phenolic compounds vary with respect to the rate at which toxicity is reduced with dilution. Some retard growth over a wide range of dilution and do not stimulate growth in very dilute solutions. Others stimulate growth when diluted slightly beyond the inhibitive concentration. This is particularly true with some of the

more commonly occurring substances such as guaiacol, veratrol, vanillic acid, veratric acid, and vanillin.

The results of the present experiments show that the four fungi studied react quite differently from one another to the various compounds. If a longer list of fungi had been included a still greater range of tolerance might have been expected. Wide differences are found between onion parasites. For instance, protocatechuic acid inhibited *C. circinans* at 1–800 and retarded growth at 1–12,800 (table III), while *A. niger* was not retarded at 1–200 (table V). These data are in accord with the fact that colored onions containing this acid are very resistant to *C. circinans* and quite susceptible to *A. niger* (**23**).

It has frequently been inferred in phytopathological studies (**5, 6, 7, 10, 16, 17, 18**) that the mere presence in the plant of free phenolic substances, usually deduced from qualitative tests, or of compounds containing phenolic residues (the soluble pigments and tannins, for instance), might be regarded as means of protection against invading organisms. That these inferences are open to serious criticism is clear from the data presented here. It appears that the deciding factors are: (a) the specific phenolic substances present, (b) the concentrations in which they occur, and (c) their toxic or stimulative effect at such concentrations on the parasite in question. It is prudent to reserve judgment on the protective rôle of a phenolic substance (or substances) until it has been isolated from the host tissue in the pure state, whereupon its toxicity to the parasite in question, over a wide range of concentration, can be studied.

V. Summary

1. The effect of twenty-one phenolic compounds upon the growth of four fungi (*Colletotrichum circinans*, *Gibberella saubinetii*, *Botrytis allii*, and *Aspergillus niger*) in Czapek's solution was studied.

2. In the phenol and phenolic acid series the position of the hydroxyl groups on the benzol nucleus is highly important in determining toxicity. Thus when the hydroxyls are arranged in ortho position to one another there is an ascending order of toxicity with increase in molecular weight: phenol, catechol, pyrogallol; while when they are in meta position to one another there is a descending order of toxicity with increase in molecular weight: phenol, resorcinol, phloroglucinol.

3. Some compounds tend to retard growth through a long series of dilutions beyond the inhibitive concentration, namely, phenol, catechol, salicylic acid. Others stimulate growth promptly in dilutions beyond the inhibitive concentration: guaiacol, meta-hydroxy benzoic acid, veratric acid, vanillic acid, and protocatechuic aldehyde.

4. Although with a few exceptions the organisms assume an increasing order of tolerance to each compound tested as follows: *C. circinans*, *G. saubinetii*, *B. allii*, *A. niger*, nevertheless there are often much wider differences among them in degree of tolerance to one compound than to another.

5. From these studies it is clear that many phenolic substances with a wide distribution in the plant kingdom exhibit little or no toxicity to certain parasitic organisms. Consequently the mere presence of phenolic substances in a host plant does not warrant the conclusion that they play a rôle in the resistance of that host to a given parasite or parasites. Toxic phenolic substances might be present in concentrations so low that their inhibitory effects are negligible, and they might also be present in concentrations that have a stimulative effect. When a phenolic substance with a specific toxicity toward a given organism is present in the host in an appropriate concentration, it may be regarded as part of the disease resisting mechanism of that host.

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GENETICAL AND CYTOLOGICAL STUDY OF SPECIES HYBRIDS OF ASIATIC AND AMERICAN COTTONS

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(WITH TWENTY-TWO FIGURES)

Introduction

The cultivated species of *Gossypium* have been divided into two groups, the Asiatic and the American (**29, 16**). Each of these groups includes several species; for example, of the common species *G. arboreum*, *G. nanking*, and *G. herbaceum* belong to the Asiatic group while *G. hirsutum*, *G. barbadense*, and *G. peruvianum* belong to the American group. Within each group the species cross readily but crosses between the different groups have been found difficult to make. As early as 1872, CLARK (cited by WATT **24**) reported the latter as "not possible." Recently some successful crosses between Asiatic and American cotton species have been reported (**25–27, 9**), but the F_1 plants showed complete sterility either when selfed or when back-crossed to the parental species. The reason for this is not yet known.

NIKOLJAVA (cited by ZAITZEV **25, 27**), DENHAM (**8**), and BANERJI (**2**) reported different chromosome numbers in Asiatic and American species of *Gossypium*. The Asiatic group has 13 haploid or 26 somatic chromosomes, while the American group has 26 haploid or 52 somatic chromosomes. This throws new light on the problems of the difficulty of making successful crosses between Asiatic and American species and of the sterility of their hybrids, but the cytology of the sterile F_1 hybrids was not reported until 1931.

The writer became interested in problems pertaining to the interspecific hybrids between Asiatic and American cottons and started this work in June, 1931. The results obtained in the past two years are reported in this paper.

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Literature review

1. INTERSPECIFIC CROSSES OF COTTON.—ZAITZEV (**25–28**) obtained two hybrids between *Gossypium herbaceum* and *G. hirsutum*. DESAI (**9**) obtained one hybrid plant from crosses between these two species. They both used *G. herbaceum* as the female parent. NAKATOMI (**18**) reported two successful hybrids between *G. hirsutum* and *G. herbaceum* and one between *G. barbadense* and *G. herbaceum*. HARLAND (**15**) reported two hybrids between *G. barbadense* and *G. arboreum* var. *sanguinea*. These two investigators also used Asiatic cotton as the female parent, but both met with no success. C. C. FENG (personal communication) obtained one hybrid between *G. hirsutum* (variety King) and *G. nanking* (variety Chinese White) while working at Georgia State College of Agriculture in 1920. ZAITZEV (**28**) described five natural hybrids between *G. herbaceum* and *G. hirsutum*. The present writer saw two natural hybrids between American and Asiatic cottons (most probably between *G. hirsutum* and *G. nanking*) in the summer of 1930 at the Experiment Station of National Central University, Nanking, China. In all these cases the hybrid plants showed considerable vigor and were self-sterile. When back-crossed to parental or other species, they were also sterile except in one case reported by HARLAND (**15**), who obtained eight plants as a result of back-crossing to *G. barbadense* with the pollen grains of the hybrids. SZYMANEK and GAVAUDAN (**21**) reported a fertile hybrid between *G. herbaceum* and *G. hirsutum*, and SZYMANEK (**20**) reported a fertile hybrid between *G. herbaceum* and *G. vitifolium*, but these results seem doubtful.

CYTOLOGICAL STUDIES ON COTTON.—CANNON (**6**) reported 28 as the haploid chromosome number of a hybrid between Sea Island cotton (*G. barbadense*) and Upland cotton (*G. hirsutum*). BALLS (**1**) reported 20 as the haploid chromosome number of an Egyptian variety (*G. peruvianum*). NIKOLJAVA (cited by ZAITZEV **25, 27**) found that the somatic chromosome number is 26 in Asiatic cotton and 52 in American cotton. DENHAM (**8**) reported that Old World cottons have 13 haploid chromosomes, one being larger than the others; and that New World cottons have 26 haploid chromosomes with two larger than the others. BEAL (**4**) determined the haploid chromosome number of three American species as 26. BANERJI (**2**) confirmed the numbers given by DENHAM. BARANOV (**3** = BARANOV,

MICHAILOVA, ELLENHORN, 1930; cited by EMME **11**) found that the Old World cottons have 26 somatic chromosomes and the New World cottons have 52. The chromosome morphology was briefly described. In the F_1 hybrid of *G. hirsutum*×*G. herbaceum* BARANOV observed 13 bivalents and 13 univalents, the latter passing irregularly to the poles. ZHARBIN (cited by EMME) also counted 13 bivalents and 13 univalents in a *G. hirsutum*×*G. herbaceum* hybrid. NAKATOMI (**18**) reported the haploid chromosome number of *G. herbaceum* to be 13 and that of *G. hirsutum* and *G. barbadense* to be 26. He described the chromosome behavior in microsporogenesis of F_1 hybrids between Asiatic and American cottons. SZYMANEK and GAVAUDAN, and SZYMANEK reported variable chromosome numbers, for example, 10–13 haploid for Asiatic species and 20–26 diploid for American species. EICHHORN (**10**) published a brief note on somatic mitosis of *Gossypium* but no chromosome number was given. LONGLEY (**17**) reported 13 as the haploid chromosome number for Asiatic cottons and 26 for American cottons. He also made a fairly detailed description of the microsporogenesis of an Asiatic×American F_1 hybrid obtained by an accidental cross. SKOVSTED (**19**) reported 26 somatic chromosomes for Asiatic species. On the basis of chromosome configurations in a triploid Asiatic plant and other evidence, SKOVSTED suggested that "the Asiatic cottons ($n=13$) must be polyploids." With a few exceptions (**6, 1, 20, 21**) the following points are agreed upon in these cytological studies: (1) the Asiatic species have 13 haploid chromosomes (26 somatic) and the American species have 26 haploid chromosomes (52 somatic); (2) the sterility of the Asiatic-American F_1 hybrids finds a cause in the irregular chromosome behavior in the microsporocytes during the meiotic divisions.

Materials and methods

Typical Asiatic and American cotton species were obtained from agricultural institutions of China and the United States. Two species of Asiatic cotton, *G. arboreum* L. var. *neglecta* Watt. and *G. nanking* Meyen,[2] and two species of American cotton, *G. barbadense*[3]

[2] The scientific names for the Asiatic species given in this section follow the scheme of WATT (**24**). HARLAND (**16**) suggested that there is only one species in Asiatic cotton, *G. nanking* being reduced to a variety under *G. arboreum*.

[3] According to HARLAND (**16**).

and *G. hirsutum*, were used. In each species there were one to several varieties or strains. It was not possible to make several generations of selfing before the work was started, but the varieties used appeared to be uniform.

The crosses were made under greenhouse conditions. The methods of emasculation, pollination, tagging, and bagging were those employed by C. C. FENG (**13**). One precaution was added: before pollination the stigma was examined with a 10× lens to see whether any stray pollen grains of the same flower were attached to it.

The cytological observations were made on slides prepared by the paraffin method. The somatic chromosomes were studied in root tips. Flower buds were fixed for studies of microsporogenesis, the bracts, perianth, and ovary being removed to facilitate killing. Fixation for both studies was mostly made in Nawaschin's solution. Bouin's solution was used once. Sections were cut 8–10 μ thick and stained with Haidenhain's iron-alum haematoxylin according to Sharp's schedule.

For studies of pollen tube growth, the flowers were artificially pollinated and kept in a controlled temperature room at 25° C. for 24 hours and then prepared according to the method given by BUCHHOLZ (**5**) for *Datura*. In cotton, however, there is great difficulty in removing the cortex of the style. Apparently there is no clear differentiation between cortex and conducting tissue like that in *Datura*.

Asiatic-American hybrids

A. COMPATIBILITY

To test the compatibility between Asiatic and American species, 1708 interspecific crosses were made during the summers of 1931 and 1932. The results obtained are given in table I.

The hybrid bolls obtained were generally very small and contained only one to few seeds. Out of 29 bolls obtained in the last two years, 21 contained only one seed each, five contained two each, two contained three each, and one contained five seeds. When the seed was sown not every one germinated. Some were found to contain a very small embryo or none at all, even though they possessed well developed seed coats. Out of 22 ungerminated seeds examined in the

last two years, 13 were of this type. A few seeds had an embryo, but either the radicle failed to elongate or the seeds decayed. When the plants were grown, some were identical with the female parent, showed complete fertility, and gave no indication of hybrid vigor. These plants are most probably the result of accidental contamination by the pollen grains of the same flower or of the same variety. In all cases the pollen parent carried dominant factors which made

TABLE I

RESULTS OF CROSSES BETWEEN ASIATIC AND AMERICAN SPECIES OF GOSSYPIUM

PARENTS	CROSSES MADE	BOLLS OBTAINED	SEEDS OBTAINED	PLANTS GROWN	HYBRID PLANTS
ASIATIC×AMERICAN					
G. arboreum×G. hirsutum	54	0	0	0	0
G. arboreum×G. barbadense	1	0	0	0	0
G. nanking×G. hirsutum	836	2	2	2	1
G. nanking×G. barbadense	126	3	5	4	0
Total	1017	5	7	6	1
AMERICAN×ASIATIC					
G. barbadense×G. nanking	91	0	0	0	0
G. barbadense×G. arboreum	43	0	0	0	0
G. hirsutum×G. nanking	407	21	27	10	5
G. hirsutum×G. arboreum	150	3	8	4	0
Total	691	24	35	14	5
Grand total	1708	29	42	20	6

it possible to distinguish the true hybrids. In the last column of table I, the number of true hybrids is listed.

Table I shows that hybrid plants were obtained only from *G. hirsutum*×*G. nanking* and the reciprocal cross. It cannot be stated that these two species are more compatible than others, however, since more varieties and more plants were available for these two species and naturally more crosses were made between them. NAKATOMI (**18**) obtained one hybrid plant from *G. barbadense*×*G. herbaceum*, and HARLAND (**15**) obtained two hybrids from *G. barbadense*×*G. arboreum*. Sufficient data are not at hand to determine the difference in compatibility between different species.

Considering the compatibility of reciprocal crosses between Asi-

atic and American species, the cross of Asiatic ♀ × American ♂ does seem to be less compatible. Out of 1017 crosses with Asiatic cotton as the female parent, only one hybrid plant was obtained; while out of 691 crosses with American cotton as the female parent, five hybrid plants were obtained. This is a significant difference. NAKATOMI found more successful crosses with American cotton as the female parent. HARLAND (**15**) reported no success with Asiatic cotton as the female but obtained two hybrid plants with American cotton as female. This conforms to the general results of other interspecific crosses, in which greater success is obtained when the plant with the larger chromosome number is used as the female (**22**).

The writer was fortunately successful in obtaining hybrid plants from crosses in both directions; previous workers had reported success only with crosses in one direction or the other: Asiatic ♀ × American ♂ (ZAITZEV, DESAI); American ♀ × Asiatic ♂ (NAKATOMI, HARLAND).

Another difference between the reciprocal crosses is a difference in the retention of the young bolls on the plants after pollination. When American cotton was used as the female parent the unsuccessful ovaries dropped within two weeks after pollination. In the last two years only one or two small bolls (which gave no matured seed) out of 691 crosses were retained on the plant. In the reciprocal cross, with Asiatic cotton used as the female, 212 bolls out of 1017 crosses were retained on the plants and developed nearly to the normal size. These bolls did contain some weak fiber but no well developed seed; hence they could not be considered as successful crosses. This phenomenon has been mentioned by ZAITZEV (**27, 28**) and NAKATOMI (**18**). DESAI'S (**9**) use of retention of bolls as a criterion of success in an Asiatic-American cross is inaccurate.

In order to determine whether or not the low percentage of success in Asiatic-American species crosses is due to slowness of pollen tube growth, observations were made by methods used by BUCHHOLZ in *Datura*. Within 24 hours after pollination the pollen tubes of American cotton were seen traveling down to the base of the style of Asiatic cotton. When Asiatic pollen grains were placed on the stigma of American cotton, the pollen tube growth also has been followed to the base of the style. Sections of the ovary have

not been studied to trace the later development. The observations suggest that the low percentage of success in crosses between Asiatic and American species is due not to the slowness of foreign pollen tube growth, but to something else, probably an actual incompatibility of gametes.

B. Hybrid vigor of F_1 plants

Only three hybrid plants obtained from crosses made in 1931 have been studied. These were named hybrids A, B, and C. Hybrid A was the offspring of a cross with Asiatic cotton as the female parent,

TABLE II

VEGETATIVE CHARACTERS OF F_1 HYBRIDS COMPARED WITH THEIR PARENTS

Plant	Height (cm.)	Number of nodes	Length of internodes (cm.)	Basal circumference of stem (cm.)
Asiatic ♀	93	20	4.65	2.3
American ♂	48	22	2.18	2.6
Hybrid A	236	40	5.90	4.2
American ♀	49	22	2.23	2.5
Asiatic ♂	99	33	3.00	1.6
Hybrid B	203	39	5.21	2.9
American ♀	49	24	2.04	2.5
Asiatic ♂	99	25	3.96	2.5
Hybrid C	151	38	3.97	3.2

while B and C were from crosses where American species were used as the female parents. These were planted on February 15, 1932. Some measurements of vegetative characters made on November 20 of the same year are given in table II.

The three hybrid plants exceed their parents in each character listed. Hybrid A was more vigorous than the other two, suggesting that the reciprocal crosses may not give equal degrees of hybrid vigor. The hybrid obtained with Asiatic cotton as the female parent was more vigorous than those obtained with American cotton as the female parent. More such crosses are needed before it can be stated whether this is generally true or not.

Hybrid vigor of the F_1 plants was reported by all the previous

workers who obtained Asiatic-American cotton hybrids. The increase in height is most conspicuous. In this case the increase in height is due to increased number of nodes as well as to an increase in the length of internodes, and not simply to an increase in the length of internodes as reported by WARE (**23**) in interspecific crosses with the American group.

C. CHARACTERS IN F_1 HYBRIDS

The six hybrid plants obtained from the 1931 and 1932 crosses between *G. nanking* and *G. hirsutum* were studied. Two varieties of

TABLE III

CHARACTERISTICS OF G. NANKING AND G. HIRSUTUM AND THEIR F_1 HYBRIDS

CHARACTERISTICS	G. NANKING	G. HIRSUTUM	HYBRIDS
1. Hair coating	Short and long hairs	Long hairs only	Long hairs only
2. Bract base	United	Free	United
3. Bract teeth	Short	Long	Long
4. Extra floral nectaries	Absent	Present	Absent
5. Calyx teeth	Short	Long	Long
6. Peduncle position	Drooped	Erect	Intermediate
7. Leaf position at night	Vertical	Horizontal	Vertical
8. Leaf lobe	Narrower	Broader	Intermediate
9. Petal color	Bright yellow	Cream	Dilute yellow
10. Petal spot	Deep red	None	Faint red
11. Anther color	Yellow	Cream	Yellow
12. Boll locks	3–4	4–5	3–4

G. hirsutum (Acala and Trice) and four varieties of *G. nanking* (Million Dollar, Paoshan Whiteseed, Kwangsi Fuzzyseed, Chinting Large Yellowflower) entered into these crosses. The two varieties of *G. hirsutum* are identical in botanical characters and the four other varieties are identical also. Such characters provided in other varieties, as red leaf, small flower, white flower, red flower, were not utilized. The six hybrid plants showed the same characteristics and therefore may be studied collectively. The characters observed are summarized in table III.

Among these six hybrid plants one was obtained from a cross using Asiatic cotton as female while five were from crosses using American cotton as female. Their characters are the same. This shows that

reciprocal crosses give the same results even in crosses between species from different groups. Since these F_1 plants are completely sterile, no further study of inheritance in F_2 can be made.

Sterility in F_1 hybrids

A. Self-sterility

From June 1932 to January 1933, 227 flowers of the three hybrid plants have been selfed: 103 on hybrid A, 58 on hybrid B, and 66 on hybrid C. Not a single flower matured a boll, the young bolls falling off within two weeks after selfing. After January, 1933, some young bolls were retained on hybrid C, but these contained no developed ovules. Selfing hybrids D, E, and F also met with no success. This showed complete sterility on selfing, and confirmed the results of previous workers.

B. Cross-sterility

Back-crosses to the parental species were made in both directions. One hundred and fifty-two flowers of the hybrid plants (73 on hybrid A, 49 on hybrid B, and 30 on hybrid C) were pollinated either by American or Asiatic species. One boll containing one matured seed resulted from pollinating hybrid C with *G. hirsutum*. This seed contained a good embryo but unfortunately did not germinate. Two other bolls resulting from pollinating hybrid C with Asiatic cotton were retained on the plant for 92 and 86 days respectively, but neither boll contained any well developed seed.

Two hundred and ninety-eight flowers of American and Asiatic species (212 on American and 86 on Asiatic) were pollinated with pollen from the hybrid plants. One boll containing six seeds was formed as a result of pollinating *G. hirsutum* (American) with hybrid C. These six seeds were planted in January, 1933, and five plants were grown. The plants were identical with *G. hirsutum* in every respect and were most probably the results of contamination by the pollen of the same species.

C. Pollen abortion

The pollen grains of the hybrid plants are very irregular in size. The pollen grains of *Gossypium* are spherical in shape. Measure-

ments of diameters of the pollen grains of the parental species as well as of the hybrids were made in the summer of 1932. The results are given in table IV.

These figures (table IV) are based upon 100 measurements in each case and the unit is the micron. The pollen grains were killed by BOUIN's solution[4] to avoid bursting, and measurements were made under a microscope. The pollen grains of the parental species are very uniform in size, as shown by the low coefficient of variability.

TABLE IV

POLLEN GRAIN SIZE AND VARIABILITY OF ASIATIC AND AMERICAN SPECIES AND THEIR HYBRIDS

PLANT	MEAN	RANGE	STANDARD DEVIATION	COEFFICIENT OF VARIABILITY
G. hirsutum.....	100.70±0.28	88.0–110.0	4.09±0.17	4.06±0.19
G. nanking.....	87.79±0.46	71.5– 99.0	6.88±0.33	7.88±0.38
Hybrid A.....	74.42±1.00	44.0–132.0	14.84±0.71	19.94±0.99
Hybrid B.....	82.72±1.59	33.0–143.0	23.54±1.12	28.46±1.46
Hybrid C.....	78.60±1.22	33.0–126.5	18.06±0.86	22.98±1.15

The pollen grains of *G. nanking* are smaller than those of *G. hirsutum*. This may be due to the fact that the pollen grains of tetraploid plants are usually larger than those of diploid plants, although this rule does not apply universally. For example, the mean diameter of *G. barbadense*, another tetraploid species measured by the writer, is 113.41±0.31 μ. The mean sizes of pollen grains of the three hybrid plants are even smaller than that of the Asiatic parent. The great irregularity is revealed by the range in size. The smallest size

[4] As this paper was being completed, attention was called to a paper by FERGUSON and COOLIDGE (14) on methods of studying the pollen grains of *Petunia*. They denounced the value of an aqueous medium in pollen grain studies because it changes the shape and size of the pollen grains. Since the pollen grain of cotton is spherical there is no distortion in shape as in the ellipsoidal *Petunia* pollen grain. For the size of the grain the writer found a 5% decrease in the diameter of the pollen grains of *G. hirsutum* and *G. nanking* when they were mounted in Bouin's solution as compared with those mounted in air. The coefficient of variability is nearly the same. This indicates that very little error is introduced when determining the size of cotton pollen mounted in Bouin's solution. Since material is not available, the variation of pollen grain size in the hybrids has not been checked.

attained was only about one-third of the mean diameter, and the largest ones were even larger than those of the American parent. They showed a high standard deviation and coefficient of variability as compared with those for the parental species.

When pollen grains of the parental species were placed in water on a slide they burst vigorously and discharged their contents. When pollen grains of the hybrid plants were mounted in water very few of them burst, and even then they discharged only a small amount of contents. Such a phenomenon has been reported by ZAITZEV (**28**). The irregularity in pollen grain size has been observed by ZAITZEV (**28**), NAKATOMI (**18**), and LONGLEY (**17**).

One characteristic of the hybrids not mentioned by previous workers is non-dehiscence of the anthers. The anthers of the normal cotton plants burst in the morning as soon as the flowers opened. The anthers of the sterile F_1 hybrids generally dehisced later than those of the normal plants. Some anthers were shrunken in appearance and failed to dehisce. In winter when the plants were kept in a cool greenhouse (55°–65° F.) there was a high percentage of flowers showing non-dehiscence of anthers.

D. OVULE ABORTION

The normal ovules are white and fleshy. A few days after pollination the beginning of the development of fibers on the seed coats is apparent. The ovules of the sterile F^1 hybrids were shrunken, dry, and black; moreover, no fibers developed on the seed coats even though the bolls had been retained on the plant as long as three months. The ovules apparently do not develop at all. Comparing the retained bolls on the hybrid plants (*cf.* under A and B of this section) and those on the Asiatic species (*cf.* under A of section IV), the development of fibers and seed coats of the unfertilized Asiatic ovules was much more advanced than that in the hybrid plants. This indicates inability of the sterile ovules to develop.

Non-functional pollen grains and ovules were reported by most of the workers dealing with Asiatic-American hybrids. From DESAI'S (**9**) statement that "The anthers appeared quite healthy and both the pollen grains and ovules did not show any obvious defects," it is doubtful whether he was dealing with a hybrid.

Chromosome numbers of parental species

The somatic chromosomes of *G. nanking* and *G. hirsutum* were studied in root tips. *G. nanking* possesses 26 somatic chromosomes (fig. 3) and *G. hirsutum* 52 (fig. 4). This confirms the results of the Russian workers (NIKOLJAVA, cited by ZAITZEV **25, 27;** BARANOV **3**). The chromosomes of *G. nanking* are easily counted because the number is not large and the individual chromosomes are well separated. The chromosomes in *G. hirsutum* are rather difficult to count because of the large number and the fact that the nucleus is only a little larger than that of *G. nanking*, which makes the chromosomes much more crowded. The diameter of a nuclear plate of *G. nanking* is about 10 μ while that of *G. hirsutum* is about 12 μ. DENHAM (**7**) gave the somatic chromosome number of three varieties of American cotton as "circa 52" because he met with the same difficulty. SZYMANEK and GAVAUDAN'S (**21**) report of 20–26 as the diploid chromosome number for *G. hirsutum* is probably incorrect. The writer never found such a small number in the root tips of *G. hirsutum*.

There are striking differences in size and shape between the chromosomes of the complement in each species. The longest chromosomes are three or four times as long as the shortest ones. Chromosomes with nearly equal arms and with unequal arms were observed. Some chromosomes appeared rod-shaped, probably owing to the fact that one of the two arms is very short. At the present time knowledge of chromosome morphology is not sufficient to enable one to distinguish the chromosome complements of any species of *Gossypium*.

The chromosome number of *G. nanking* was also determined in microsporocytes in several varieties. Figures 1 and 2 give two examples. Figure 1 was taken from a metaphase of the first division and figure 2 was from a metaphase of the second division. The haploid number is 13; this confirms the results of the previous workers. The 13 chromosomes stand out clearly and there can be no doubt as to the exact number. SZYMANEK and GAVAUDAN'S count of 10–13 for *G. herbaceum* is doubtful.

The different sizes of chromosomes at the metaphases of the meiotic divisions are not very evident, as shown in figures 1 and 2.

This agrees with the observations of BANERJI (**2**). Although DENHAM (**8**) claimed that one chromosome is larger than the others, his figures (**8,** p. 435) are not convincing.

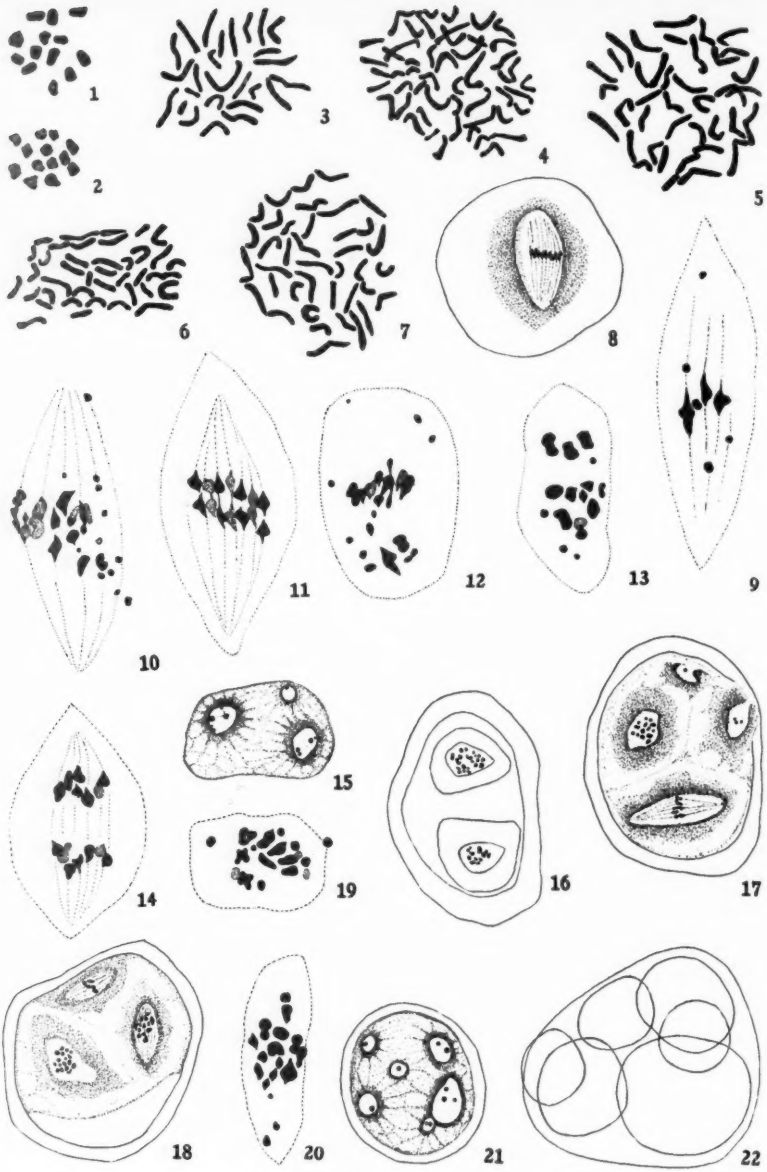
The haploid chromosome number for *G. hirsutum* was not determined from microsporocytes in this work.

Chromosome behavior in F_1 hybrids

The somatic chromosomes of the three hybrid plants A, B, and C have been studied in root tips. These contained 39 chromosomes, which is the summation of the haploid 26 from the American parent and the haploid 13 from the Asiatic parent. There is no difficulty in making counts, because the nuclear plate is nearly as large as that of American cotton and the chromosomes are not too crowded.

The following description of chromosome behavior in microsporogenesis was drawn chiefly from observations on hybrid A, which was the result of a cross using *G. nanking* as the female and *G. hirsutum* as the male parent. The chromosome behavior in meiosis in the other two hybrid plants, B and C, was similar to that described later in this paper.

The chromosome behavior before the first metaphase has not yet been traced. At metaphase of the first division the chromosomes may all lie in the same equatorial plane (fig. 8), although in most cases some of the univalents remain away from this plane (fig. 9). In polar view the bivalents and univalents can be distinguished by their sizes; the bivalents are much larger than the univalents. This difference is most clearly seen in the side view, where the univalents are small and round and the bivalents large and irregular, as if they were pulled by the spindle fiber attachment (fig. 9). The number of bivalents is generally 13 (fig. 10). The greatest irregularity comes at the anaphase, when the bivalents and univalents are scattered throughout the spindle. The univalents are distributed to the poles at random without splitting. Some of the bivalents disjoin, but others, often a large number, move toward the poles without any sign of disjunction (fig. 10); in normal plants the disjunction at anaphase is quite regular, as shown in figure 11. In a later anaphase stage, when two groups of chromosomes are pulling apart, the univalents and non-disjoined bivalents are clearly seen in each group



FIGS. 1–22.—Legend on opposite page.

and the two groups often contain different numbers of chromosomes (figs. 12, 13); in normal plants no such irregularity is seen (fig. 14). Some of the scattered chromosomes are excluded from the two daughter nuclei and form micronuclei at the end of the first division (fig. 15). There may be one to several micronuclei in interkinesis.

At metaphase of the second division the two equatorial plates frequently show different chromosome numbers (fig. 16). This is the expected result of the unequal distribution of chromosomes observed at the first division. The large bivalents and the small univalents are also clearly seen in the equatorial plates (figs. 16, 19). At this stage there are generally two major spindles; the spindles of the micronuclei are not clear (fig. 17). In other cases, however, a third spindle is conspicuous (fig. 18). In side view of the second metaphase all the chromosomes may be arranged normally in an

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FIGS. 1–22.*—Fig. 1, first metaphase in microsporocyte of *G. nanking*, polar view, showing 13 chromosomes; ×1650. Fig. 2, second metaphase in same, showing 13 chromosomes; ×1650. Fig. 3, somatic metaphase of *G. nanking*, showing 26 chromosomes; ×2500. Fig. 4, somatic metaphase of *G. hirsutum*, showing 52 chromosomes; ×2500. Fig. 5, somatic metaphase of *G. nanking*×*G. hirsutum*, showing 39 chromosomes; ×2500. Figs. 6, 7, same, showing 39 chromosomes; ×2500.

FIGS. 8–22 (with the exception of figs. 11 and 14) are of microsporocytes from one hybrid plant, *G. nanking*×*G. hirsutum:* Fig. 8, first metaphase, side view, showing regular arrangement of chromosomes in equatorial plane; ×650. Fig. 9, same, showing bivalents and univalents (bivalents are in equatorial plane but some of the univalents are not showing on this plane); ×1650. Fig. 10, early anaphase of first division, showing 13 bivalents and 13 univalents; also disjunction and non-disjunction of bivalents and irregular distribution of univalents; ×1650. Fig. 11, early anaphase from a normal *G. nanking* plant, showing normal disjunction of bivalents; ×1650. Figs. 12, 13, late anaphases in hybrid, showing non-disjoined bivalents going toward each pole; ×1650. Fig. 14, late anaphase from a normal *G. nanking* plant, showing normal distribution of chromosomes; ×1650. Fig. 15, interkinesis in hybrid, showing micronucleus in addition to two daughter nuclei; ×650. Fig. 16, second metaphase, polar view, showing different chromosome numbers in two equatorial planes; ×650. Fig. 17, second metaphase, showing one major spindle in side view, another in polar view, and two micronuclei in which spindle is not distinct; ×650. Fig. 18, second metaphase, showing third spindle with four chromosomes in addition to two major spindles; ×650. Figs. 19, 20, taken from same microsporocyte: fig. 19, second metaphase, polar view, showing different sizes of chromosomes. Larger ones are most probably non-disjoined bivalents from first division; fig. 20, early second anaphase, showing non-disjoined bivalents. Fig. 21, abnormal "quartet" containing 6 nuclei of different sizes. Fig. 22, 6 unequal-sized daughter cells formed from one microsporocyte.

* Figures drawn with aid of a camera lucida and reduced to one-half.

equatorial plate (fig. 17). At the second anaphase the distribution of chromosomes is also irregular, the chromosomes being scattered throughout the spindle. The non-disjoined bivalents may disjoin at this time or still remain intact (fig. 20). As a result of such irregular distribution of chromosomes, there are often more than four nuclei formed from one spore mother cell. These may be unequal in size owing to differences in chromosome number. The micronuclei are the result of chromosome elimination in the first or second division (figs. 21, 22). The pollen grains containing abnormal chromosome complements probably are largely, if not wholly, abortive.

A point to be emphasized here is the non-disjunction of bivalents in the first and second divisions. NAKATOMI (18) noted that in the first division "occasionally there was found one or more bivalents located in the cytoplasm without division," and that "in most cells bivalent chromosomes which were not divided in the first division were found even in the second division." The evidence of the present investigation substantiates NAKATOMI'S interpretation. LONGLEY did not mention the non-disjunction of bivalents and even gave evidence for the splitting of univalents in the first division.

The distribution of chromosomes in the second anaphase is still not fully described. NAKATOMI stated: "Most of the univalent chromosomes of each group and bivalent chromosomes were divided in homoeotypic division and the split halves of each chromosome were separated toward different poles, but some were not divided and left as dyad chromosomes." He did not make clear whether the word "some" referred to the bivalents or to the univalents. LONGLEY did not mention the chromosome behavior in the second division. Irregular quartets and abortive pollen grains were also reported in the works of NAKATOMI and LONGLEY.

Summary

1. One thousand seven hundred and eight crosses were made between Asiatic and American cotton species and six hybrid plants were obtained.

2. Out of 1017 crosses in which Asiatic cottons were used as the female parent, one hybrid plant was produced, while in 691 crosses

using American cotton as the female, five hybrid plants were obtained. This would indicate that when crosses are made between Asiatic and American cotton species better success is obtained when American cotton is used as the female parent than when Asiatic cotton is so used. Since American cotton has 52 somatic chromosomes while Asiatic cotton has 26, this agrees with the general observation that crosses using the species with the higher chromosome number as the female are more successful.

3. Studies of pollen tube growth suggest that the low percentage of success in Asiatic-American species crosses is due not to the slowness of foreign pollen tube growth, but probably to an actual incompatibility of gametes.

4. The F_1 hybrid plants obtained showed considerable hybrid vigor, especially in the height of the plant. The reciprocal crosses did not seem to show the same degree of vigor, but the evidence is not conclusive.

5. In the hybrid plants some characters were like those of the American parent, others were like those of the Asiatic parent, and still others were intermediate. The F_1 plants could be identified by these mixed Asiatic and American characters.

6. The F_1 plants were completely sterile when selfed. When pollinated with pollen of the parental species, one fruit with one seed was obtained but the seed did not grow. When the pollen grains were placed on the stigma of the American species some seeds were formed. These seeds gave rise to plants identical with the American parent. These plants were most probably the results of contamination.

7. The pollen grains of the F_1 hybrids were very irregular in size and did not burst when mounted in water. They were mostly, if not all, abortive. The ovules were also abortive, as evidenced by their shrunken appearance.

8. The somatic chromosome number of *G. nanking* is 26 and that of *G. hirsutum* is 52. The haploid chromosome number of *G. nanking* is 13.

9. The somatic chromosome number of the hybrid plants is 39. This is the sum of 26 from the American and 13 from the Asiatic

parent. The knowledge of chromosome morphology is at present not sufficient to enable one to distinguish the chromosomes derived from the two parents.

10. Meiosis in the microsporocytes of the F_1 hybrids was found to be irregular. In the first division metaphase, 13 bivalents and 13 univalents were observed. The univalents were distributed at random and part of the bivalents disjoined at the first division. The non-disjunction of bivalents was often observed, the entire bivalent moving to one pole. Some chromosomes were eliminated at the first division and formed micronuclei. In the second division more than two spindles were often observed. The chromosome distribution was also irregular at this division. There are thus formed more than four nuclei which are unequal in size and lead to the formation of irregular sized and abortive pollen grains.

11. Judging from the present status of our knowledge, the possibility of making fertile hybrids between Asiatic and American cotton species is remote unless the chromosome difference between them is removed in some way before crossing.

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COMPARATIVE STUDY OF THE DEVELOPING AND ABORTING FRUITS OF PRUNUS PERSICA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 459

THOMAS J. HARROLD

(WITH TWENTY-SEVEN FIGURES)

Introduction

A large percentage of the fruits of the peach fall from the trees before maturity, and this failure in development is a major problem among the growers of pome and stone fruits. The problem has been studied in considerable detail with the plum, the sour cherry, and the sweet cherry by DORSEY (**7**), BRADBURY (**5**), and TUKEY (**12**). With the exception of the work of DETJEN (**6**) and CONNORS (**4**), the peach has apparently been overlooked in this phase of the sterility problem.

The investigation covered the following points: (1) the situation in the peach as compared with the facts of abortion already known for the plum and cherry; (2) the careful comparison of developing and aborting fruits to aid in the discovery of the fundamental causes of this type of abortion; (3) the possible relation of the three growth periods of the peach, also recognized by other workers, to abortion and subsequent dropping of fruits.

Materials and methods

The Carman variety of peach (*Prunus persica* Sieb. & Zucc.) was used in this study. Material was collected in the College orchard at Athens, Georgia, during the season of 1933. The first collections were made about one week before full bloom, thereafter daily for a 30-day period, and then at intervals of two or three days until about three weeks before maturity. This material was of two sorts, that which was expected to develop and that which would probably not do so. Material of the developing series was secured by removing certain branches, which were taken to the laboratory and placed in water,

and then the pistils were dropped into fixing solution. Aborting material was collected on the tree as soon as it could be recognized, and by catching falling fruits in a net after jarring the tree with a padded pole. The material, both developing and aborting, was prepared by cutting away portions of the pericarp; in later stages by removing the seeds in order that fixation might be facilitated. A modification of Nawaschin's solution (BEAL 2) and formalin-acetic-alcohol were used.

Most of the sections were cut at 10 μ, and stained in iron-alum haematoxylin or Flemming's triple stain. Measurements were made from fresh and from fixed material. The figures of the diagram in figure 1 represent in each case the average of five to twenty measurements.

Determination of total abortion was made by subtracting from the number of blossoms on certain limbs the number of fruits remaining on the same limbs after the third drop.

Microscopic study

The Carman variety of peach used in this experiment dropped approximately 70 per cent of the flowers and fruits during the season of 1933 (table I). The fall of flowers and fruits occurs in waves which are designated as the first, second, and third drops. Each wave of dropping may be differentiated according to time of occurrence and by morphological characters.

Assuming that the figures of table I may be applied to the entire orchard, it is evident that pollination is not a problem.

Each wave of dropping consists of flowers or fruits which proceed normally in development until a short time before falling. This is clearly explained by BRADBURY (5), who divides the developing and aborting fruits into three groups: "Group I . . . . includes two lots: (a) those aborting fruits which will fall in the first drop and (b) the corresponding developing fruits, that is, fruits which are developing while the former are aborting. Group II is composed of fruits which were classed as developing fruits in Group I, but have become divisible into two lots: (a) aborting fruits which will fall in the second drop and (b) developing fruits. Similarly, Group III is made up of

fruits classed as developing fruits in Group II, but have become separable into two lots: (a) aborting fruits which will fall in the third drop and (b) developing fruits."

It was possible in this study to recognize aborting fruits several days before their fall.

First drop

The first wave of peach drops occurs during approximately a 3-week period beginning one to three days before full bloom. This drop consists of buds and of flowers with and without petals, containing shriveled pistils in some cases and plump pistils in others. Pericarp enlargement has not proceeded far enough to cause the shedding of the calyx cup. Fruits which develop lose the calyx cup about the middle of the first drop period (figs. 1, 2). Styles are present in both the dropped and the developing fruits. Some have two shriveled ovules as compared with the one plump ovule in all the developing fruits.

TABLE I

Carman variety; 1933

	Number of branches	Number of flowers at full bloom	Number of fruit set 2 weeks after third drop	Percentage of flowers setting fruit
Open pollinated	8	1153	378	31.9
Flowers brushed with pollen, not bagged	6	991	301	30.3

Second drop

This wave of dropping occurs during approximately a 7-day period about the fifth week after bloom. It takes place during the middle of the first period of rapid development of the pericarp and integuments (fig. 1). Dropped fruits measure from 8 to 14 mm. in length, while the developing fruits measure 20 to 27 mm. (fig. 3). Color differences are apparent, the dropped fruits differing from developing fruits in being yellowish green as compared with the darker green of the latter. Both types are devoid of calyx cups.

Third drop

This wave occurs during approximately a 7-day period about the seventh week after bloom. These fruits vary widely in size, measuring 25 to 30 mm. compared with an average length of 37 mm. in the developing fruits (figs. 1, 4). The flaccid and yellow-green appearance which characterized the second drop is also evident. The integuments have attained nearly the size found in mature fruits.

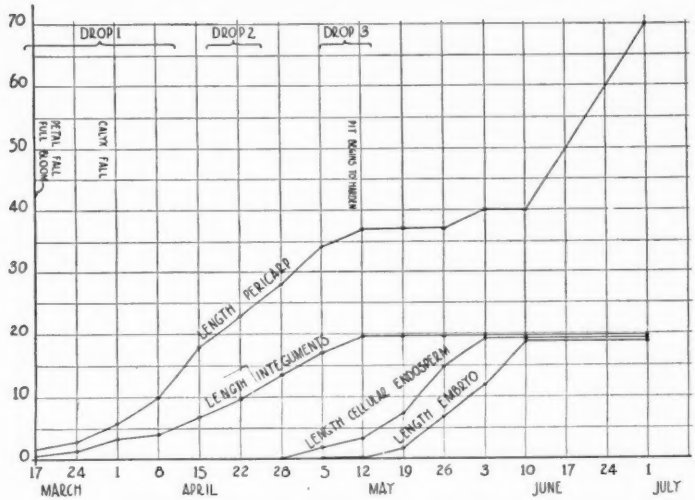


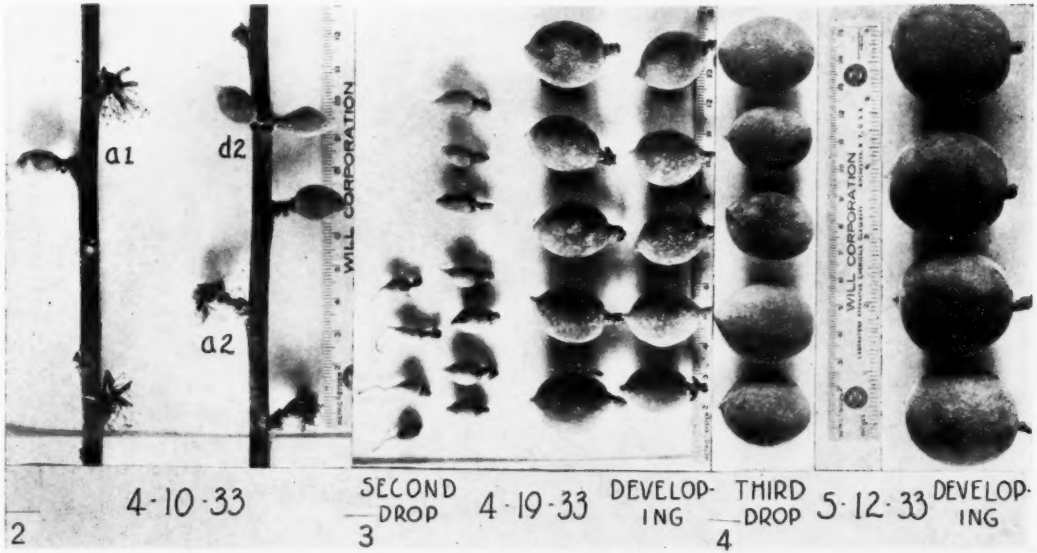
Fig. 1.—Diagram showing development of pericarp, integuments, cellular endosperm, and embryo in relation to time of drop.

Microscopic study of normal development

Correlation of parts of developing fruits with time of development is given in figure 1. This includes measurements of pericarp, integuments, cellular endosperm, and embryo. The diagram also shows the relation of each of the three drops to the developing fruits.

Pericarp

Enlargement of the pericarp of the peach does not proceed uniformly, but is divided into three stages (fig. 1). This has previously been recognized in the peach by Connors (3), Dorsey and Mc-



FIGS. 2–4.—Fig. 2, branch about the end of the first drop: *a1*, aborting first drop; *a2*, aborting second drop; *d2*, developing fruit. Fig. 3, condition during middle of second drop, showing aborting and developing fruits. Fig. 4, condition at about the end of the third drop, showing aborting and developing fruits.

MUNN (8), and LILLELAND (10); and in the sweet cherry by TUKEY (12). During the first period of eight weeks, the pericarp increased in length from 1.7 to about 37 mm., followed by a second period of four weeks in which the rate of development of the pericarp was retarded. The third period of three weeks is characterized by a final rapid enlargement of the pericarp, ending at maturity.

Pericarp enlargement is accounted for by ADDOMS, NIGHTINGALE, and BLAKE (1) as being associated with cell division during the first 30 days and subsequently with cell expansion.

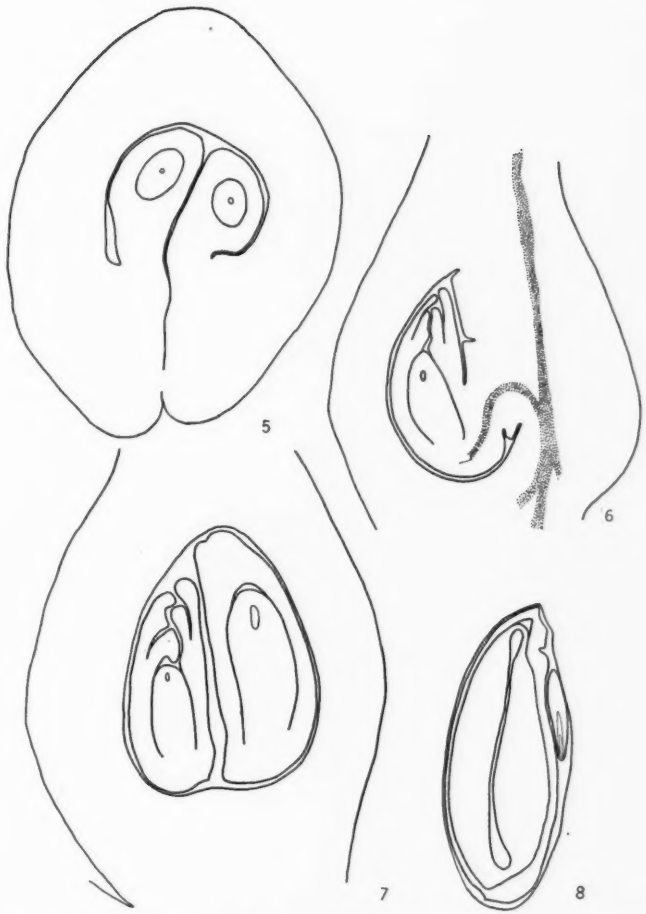
During the second period of growth (fig. 1), hardening of the inner wall of the pericarp takes place, forming the "pit."

NUCELLUS AND INTEGUMENTS

Two anatropous ovules are differentiated within a single carpel (figs. 5, 6). These two ovules develop equally until about three days before full bloom, when one of them gains the ascendancy and the other ceases to develop, so that at least a day before full bloom the latter may be recognized by its smaller size (fig. 7). The non-functional ovule is first recognized in stained sections by a peculiar transparency of the nucellus, while at the same time the gametophyte within appears normal. Degeneration involves shrinkage of the nucellus and a loss of characteristic organization by the gametophyte. The nucleoli of the latter apparently persist longer than do the other parts. These findings agree with those reported for the sour cherry (5).

The condition of a non-functional ovule at the time of full bloom is illustrated in figure 13. The latter is from the same carpel that contains the functional ovule shown in figure 25. Cessation of development of the non-functional ovule may occur at any point between the megaspore mother cell stage and the various stages in development of the gametophyte. Development of the functional ovule proceeds rapidly after full bloom, so that differences between the functional and the non-functional ovule become increasingly apparent. Figure 8 represents the relative development of the two ovules two weeks after fertilization.

The enlargement of the seed parallels the development of the pericarp during the first period of growth, so that by the close of the



FIGS. 5–8.*—Fig. 5, transverse section of pistil at time of full bloom; ×135. Fig. 6, longitudinal section of pistil at time of full bloom; ×135. Fig. 7, functional and non-functional ovules at time of full bloom; ×135. Fig. 8, relative development of two ovules two weeks after fertilization, showing elongation of embryo sac.; ×45.

* All drawings were made with the aid of an Abbé camera lucida.

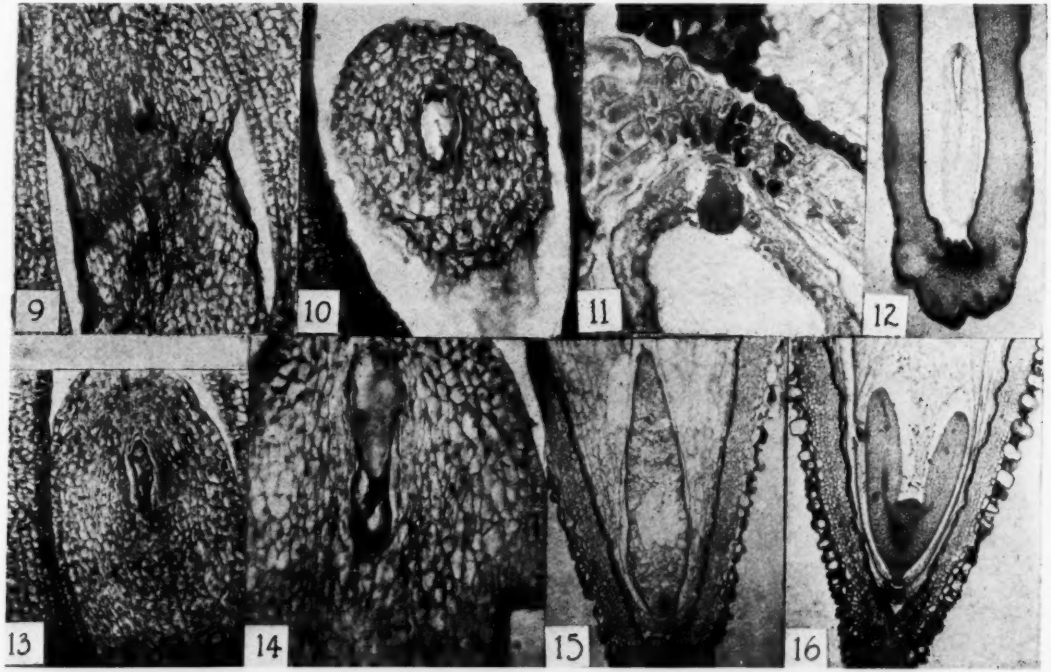
8-week period after bloom the integuments have attained a final and maximum size of about 20 mm. (fig. 1). The early maturation of the integuments has been recognized by many workers, including PÉCHOUTRE (**11**), TUKEY (**12**), DORSEY and MCMUNN (**8**), and CONNORS (**3**). The maximum size of the integuments is reached about two weeks after cellular endosperm appears and is approximately coincident with an appreciable enlargement of the embryo.

The two integuments of the peach are distinct for only about one-fourth of their length (fig. 6).

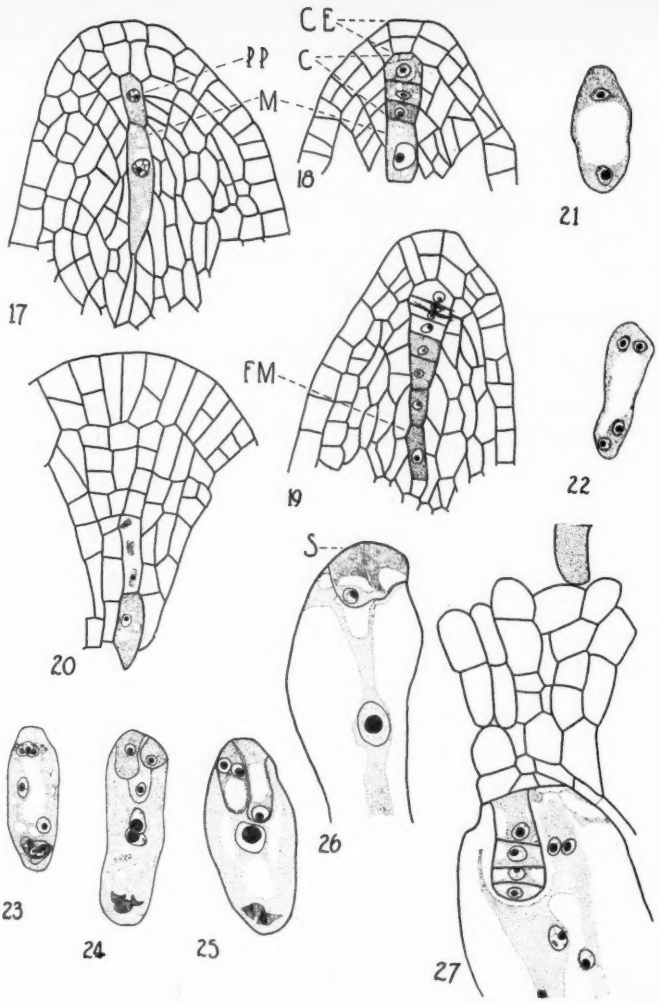
MEGAGAMETOPHYTE

The megaspore mother cell (fig. 18) is imbedded in the nucellus, being covered with three or four parietal cells and two or three layers of cells derived from the epidermis of the nucellus at the micropylar end (coiffé épidermique and calotte respectively of PÉCHOUTRE **11**). Reduction divisions resulting in a linear tetrad of megaspores (fig. 19) occur a few days before bloom. The chalazal megaspore develops and the remaining megaspores rapidly disintegrate (fig. 20). The three divisions of the functional megaspore (figs. 21, 22) follow in rapid succession, so that a day or two before full bloom the megagametophyte (fig. 23) is in the 8-nucleate stage. Maturation and migration of the two polar nuclei to the middle of the megagametophyte (fig. 24) is practically coincident with full bloom. The two polar nuclei unite (fig. 25) during the period between full bloom and fertilization. The three antipodal nuclei are short-lived (fig. 25), being well along toward disintegration at the time of polar nuclear fusion. The synergids persist for about a week after fertilization.

The embryo sac begins to elongate following the union of the polar nuclei, doubles in length by the time of fertilization, and within two weeks extends almost to the chalaza (fig. 8). The embryo sac is characteristically swollen at the chalazal end, and owing to its narrow connecting canal forms a more or less dumb-bell shaped structure. TUKEY (**12**) reports the membrane surrounding the elongated sac to be continuous and persistent. According to BRADBURY (**5**) and DORSEY (**7**), when fertilization is prevented elongation of the embryo sac takes place, extending as much as two-thirds of the distance to the chalaza.



FIGS. 9–16.—Fig. 9, portion of ovule from aborting pistil of first drop, prior to full bloom; ×400. Fig. 10, portion of ovule from aborting pistil of first drop, after full bloom; ×400. Fig. 11, embryo and endosperm development in a developing fruit at time of second drop; ×400. Fig. 12, embryo, endosperm, nucellus, and integument development in aborting fruit of second drop; ×63. Fig. 13, portion of non-functional ovule at time of full bloom (same carpel as in fig. 25); ×400. Fig. 14, portion of ovule showing primary endosperm nucleus (zygote in adjoining section) of fruit of first drop, apparently "healthy." Note shrinkage of nucellus at chalazal end; ×400. Fig. 15, portion of ovule showing early cellular endosperm, 7 weeks after full bloom: ×63. Fig. 16, portion of ovule of developing fruit at time of third drop. Note embryo and cellular endosperm; ×63.



FIGS. 17–27.—Fig. 17, portion of nucellus of ovule prior to full bloom, showing primary parietal cell (*pp*) and megaspore mother cell (*m*). Fig. 18, portion of nucellus of ovule prior to full bloom, showing coiffé épidermique (*ce*), three parietal cells (*c*), and megaspore mother cell (*m*). Fig. 19, portion of the nucellus showing four megaspores and functioning megaspore (*fm*). Fig. 20, portion of nucellus showing functioning megaspore and three disintegrating megaspores. Fig. 21, megagametophyte at 2-nucleate stage. Fig. 22, same at 4-nucleate stage. Fig. 23, same at 8-nucleate stage. Fig. 24, megagametophyte at time of full bloom. Note disintegration of antipodals. Fig. 25, megagametophyte just prior to fertilization. Note fusion of polar nuclei and their proximity to megagamete. Fig. 26, embryo sac following fertilization. Note disintegrating synergids, zygote, and primary endosperm nucleus. Fig. 27, portion of nucellus two weeks after fertilization. Note remains of pollen tube, 4-celled embryo, and numerous free nuclei of endosperm. All ×1860.

Fertilization

Double fertilization as described by Bradbury (**5**) and reported by Tukey (**12**) is found also in the peach. Evidence of fertilization was found in material in which the style had begun to fade, which was collected four days after full bloom. Prior to fertilization, the megagamete and the fused polar nucleus take positions close together (fig. 25). For a short period after fertilization the one-nucleate embryo and one-nucleate endosperm remain close together; then begins a gradual movement of the primary endosperm nucleus down the embryo sac toward the chalaza.

Endosperm

Following fertilization, the endosperm remains uninucleate for a period of nine or ten days (fig. 26). The first division of the primary endosperm nucleus takes place at a point nearly one-half of the distance to the chalaza. Following the first division there occur in rapid succession numerous divisions of endosperm nuclei, so that the embryo sac is soon lined with a peripheral layer of free-nuclear endosperm (fig. 27).

This coenocytic condition persists until about the end of the sixth week after bloom. Walls begin to appear at that time at the micropylar end of the embryo sac. Development of cellular endosperm is rapid, so that during the following five weeks it comes to extend to the chalaza. This development takes place during the period of retarded pericarp enlargement (fig. 1).

Embryo

The zygote nucleus does not divide until about 12 days after fertilization, at which time there have been many divisions of the endosperm nuclei. Embryonal development is extremely slow until about a week before the beginning of the period of retarded pericarp growth and until after considerable cellular endosperm has been formed (fig. 15). The early retardation of the embryo has been noted by Connors (**3**), and by Dorsey and McMunn (**8**); and by Tukey (**12**) in the sweet cherry. Acceleration of the embryonic growth begins about the time that the cellular endosperm has reached one-third to

one-half the length of the nucellus. Cotyledons (fig. 16) are differentiated and the embryo quickly extends almost to the chalaza, digesting cellular endosperm and nucellus before it, and completing its elongation approximately 12 weeks after bloom (fig. 1). Following the attainment of full size of the embryo, the final 3-week period of pericarpic enlargement occurs.

Microscopic study of aborting fruits

First drop

The buds which fall before, during, and after full bloom have pistils in various stages of development and degeneration, which have apparently proceeded normally up to a point between the megaspore mother cell and the early gametophyte stages. Growth then stops and degeneration begins. Buds which fall in the first drop contain pistils in which both ovules have ceased to develop. The degeneration of the second ovule closely resembles that of the first. This check in development of both ovules before opening of the flower is recognized in the plum by Dorsey (**7**) and in the sour cherry by Bradbury (**5**).

Flowers which fall with expanded petals contain ovules which have proceeded still further in development, usually being found in the two-, four-, or eight-nucleate stages of the megagametophyte. These megagametophytes evidence nuclear and cytoplasmic degeneration such as has been described for the non-functional ovule.

Flowers without petals also fall in the first drop. As a rule ovules of these pistils indicate that development has proceeded to a still further degree than that which has been described for those with petals. In this group are found ovules in which degeneration apparently began at the mature megagametophyte stage (fig. 10). Other ovules in this group give evidence of fertilization having taken place (fig. 14). In contrast to this, Dorsey (**7**) accounts for the entire drop on the basis of pistil abortion. The conditions shown in figures 10 and 14 are described by Bradbury as being found in the second drop, in cases in which an embryo is not present. In certain cases (fig. 14) there is evidence of a shrinking of the nucellus at the chalazal end, while the embryo sac is apparently normal.

Second drop

Fruits which fall in the second drop are among those which were considered as developing during the course of the first drop. Growth has proceeded to a further degree, the general relations of which are shown in figure 1. This drop occurs during the time of free-nuclear endosperm and early embryonal stages of developing fruits. The condition of embryo and adjacent endosperm is shown in figure 11, while the conditions of the corresponding drop is shown in figure 12. There is apparently no lack of development of integuments shown in the dropped fruits, the chief differences between the developing and aborting fruits being found in the nucellus, endosperm, and embryo.

Dorsey (7) reports this drop to be a result of lack of fertilization, while Bradbury, on the contrary, finds that in some cases fruits of the second drop do have embryos and in other cases do not. Fruits in which pollination was prevented were found by both Dorsey and Bradbury to fall in the first and second drops.

Third drop

The embryos from fruits which fall in the third drop exhibit no apparent structural differences from those of developing fruits (fig. 16). Dorsey (7), Bradbury (5), and Tukey (12) report that fruits which fall in the third drop contain apparently normal embryos which, for some reason, have ceased development, size differences being the only observable ones between developing and aborting embryos. Fallen fruits contain seeds in which the integuments have begun to turn brown at the chalazal end, in contrast to no browning of those of seeds of developing fruits. This condition of browning is reported by Dorsey (7) as being evident both in the second and the third drops of the plum.

Discussion

The only apparent difference between aborting fruits and developing fruits is that growth has stopped in one and has continued in the other. Thus differences between developing and aborting fruits at the time of falling exhibit mainly degrees of development, and in some cases degeneration of normal parts. At the exact moment of cessation of development, there should be no structural differences

between fruits that are to develop to maturity and those that are to abort. In the first drop is found cessation of development at stages leading up to the gametophyte, also of gametophytes and of zygote and primary endosperm nucleus; and in the second and third drops a cessation of development of embryo and endosperm, which agrees in the main with the report of BRADBURY (**5**). This differs from the report of DORSEY (**7**) in that the first drop includes more than only "aborted pistils" and that the second drop results from more than "lack of fertilization," but agrees in regard to the third drop.

Granting the fact that development proceeds normally until the time of abortion, a diagram (fig. 1) may aid in the prediction of the approximate time at which development ceases, and the degree of development of the parts of each dropped fruit. Thus if the fruits of the second drop measure 8 to 14 mm. in length, it is reasonable to expect that growth stopped at about the time that most of the fruits had attained this measurement, which the chart shows to be approximately April 6–13. It is inferred also that these fruits fell approximately April 17–26. According to the diagram, the fruits which developed until April 6–13 possessed free-nuclear endosperm and a slight degree of embryonal development. The material shows this to be true. Likewise in the third drop the chart predicts cellular endosperm and additional embryonal development, which also is confirmed by the material.

TUKEY (**13**) has demonstrated that if the embryos from an early variety of sweet cherry, which does not produce viable seed, are placed under the proper conditions of artificial culture they continue to develop. As described by him, the embryos of the fruits which were checked in development before maturity of the embryo were in many ways similar in condition to the embryos contained in fruits of the third drop of the peach, the chief difference being that TUKEY (**12, 13**) was working with aborted embryos of developing fruits and not aborted embryos of dropping fruits. If it were possible to demonstrate that embryos of fruits falling in the third drop could be artificially cultured, then it would appear that the cause of the third drop was not due to the fact that the embryos ceased to develop, but rather as a result of some other factor.

DORSEY (7) observed in the second drop of the plum that the integuments begin to turn brown at the chalazal end, and that there is a shrinkage of the nucellus, before the pistil falls, and in the third drop that the nucellus is shriveled and "early browning of the seed coats soon follows suppression of the embryo." BRADBURY (5) found associated with cessation of development in each of the three drops a shrinking of the nucellus. TUKEY (12) inferred that inasmuch as in the early-ripening non-viable seed varieties of sweet cherry the embryos are often proportionately larger than the nucellus and integuments (a condition not found in viable seed varieties), perhaps "a check in the development of integuments and nucellus precedes a check in the development of the embryo."

Cases such as shown in figure 14 represent a situation in which apparently degeneration of the nucellus in the region of the chalaza precedes any change from normal in the embryo sac. These observations lead toward the idea that degeneration, and possibly cessation of development in fruits which will drop, begins at the chalazal end of the ovule. The seeds of fruits which fall in the second and third drops are brown at the chalazal end, the browning extending toward the micropylar end as degeneration proceeds. These observations suggest that in the course of abortion development stops first in the region of the chalaza and extends from there to the other parts of the seed and fruit. Such a disorder in the chalazal region would disarrange the vascular system, and might mean that development of the entire fruit stops because of a failure of conduction. This suggests that possibly the fruit falls, not because the embryo aborts but because these events are occasioned by an upset of the vascular system in the region of the chalaza.

Summary

1. The development of the megagametophyte and fruit of the peach agrees with facts established for other members of the genus *Prunus*.

2. The dropping of pistils occurs in three distinct waves during the first growth period, and each wave bears a constant relation to the developing pistils.

3. The aborting fruits differ from developing fruits mainly in degree of development of normal parts.

4. The possibility is indicated that a disorder in the region of the chalaza may occur prior to cessation of development of gametophyte or of embryo, resulting in subsequent degeneration of the seed and fruit.

The writer acknowledges the aid of members of the Department of Botany of the University of Chicago, under whose direction the work was conducted, and of his colleagues at the University of Georgia.

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CYTOLOGICAL EFFECTS OF A GENE IN DATURA WHICH CAUSES DYAD FORMATION IN SPOROGENESIS

SOPHIA SATINA AND A. F. BLAKESLEE

(WITH THIRTY FIGURES)

Introduction

Several genes responsible for disturbances connected with the reduction division have been found in plants from nature (**1, 2, 3, 4**). In some of the cases reported, only the pollen mother cells were affected. Thus BEADLE (**2**) found a failure of cytokinesis in maize during meiosis, but this was confined to the microsporocytes. In the other cases described (**1, 3**) a recessive gene caused disturbances in the meiosis of both mega- and microsporocytes.

The meiotic irregularities discussed in the present paper were discovered by Mr. S. HOROWITZ in the summer of 1931, during his brief stay at the Department of Genetics while in this country as a Guggenheim Fellow. In a group of F_2 *Datura* cultures from radium-treated pollen, HOROWITZ found one segregating culture of 35 plants which had eight individuals with the young microspores in dyads instead of in tetrads. The pollen grains (mature microspores) of such plants were twice the size of those of normal diploids. As was proved later, this formation of dyads was due to a recessive gene which was named "dyad" (dy). Dyad plants were recognizable by external appearance, since they were more open in habit of growth.

The chromosomes of dyad plants were found to form 12 normal bivalents at the metaphase stage of the first meiotic division. In the pollen grains, however, there were 24 instead of the 12 chromosomes normally present. Since HOROWITZ was unable to carry further his study of the dyad type, the investigation was continued by the present writers.

The dyad gene has been continued through four generations by female backcrossing a dyad to a normal and later recovering dyad plants in the next generation by selfing the heterozygous parents.

When selfed, a 2n dyad plant gives rise to 4n dyads. The latter produces seed with difficulty, but a single malformed plant in the offspring had 72 chromosomes in somatic tissue, showing that this individual was 6n in chromosomal constitution.

A later report will be made of the genetic location of the gene involved and of other matters relating to the breeding behavior. The present paper gives the results of a detailed cytological study of both mega- and microsporogenesis, and also of both female and male gametogenesis.

Methods

The following fixatives were found by testing to be most satisfactory:

1. Lewitzky's fixative, consisting of 1% chromic acid (5 parts) and 10% formaldehyde (4 parts).
2. Modified Carnoy, consisting of 85 cc. of 70% alcohol, 5 cc. glacial acetic acid, and 5 cc. commercial formaldehyde.
3. Belling's iron acetocarmine.

Lewitzky's fixative gives the best results when it is used for studying details of early stages. This is true both for sectioned material and for smears. Modified Carnoy is generally good for all stages. Megasporogenesis and female gametogenesis had to be studied from sectioned material. Sections were cut from 5 to 10 μ in thickness and stained with Haidenhain's iron haematoxylin. Microsporogenesis and male gametophytes were studied both from sectioned material and from smears. Smears were stained with Haidenhain's iron haematoxylin and destained with picric acid as recommended by TUAN (8). Frequent use of Belling's iron acetocarmine method also was made. For studying the mitotic division in the young microspores (pollen grains), chloral hydrate was added (roughly one drop of 10% solution of chloral hydrate to one drop of iron acetocarmine). This treatment cleared the cytoplasm from inclusions which otherwise obscured the chromosomes. All the drawings in this paper which represent stages in microsporogenesis and in male gametogenesis were from acetocarmine preparations, except figures 15 and 19, which were from material fixed in Lewitzky and Carnoy respectively.

Observations

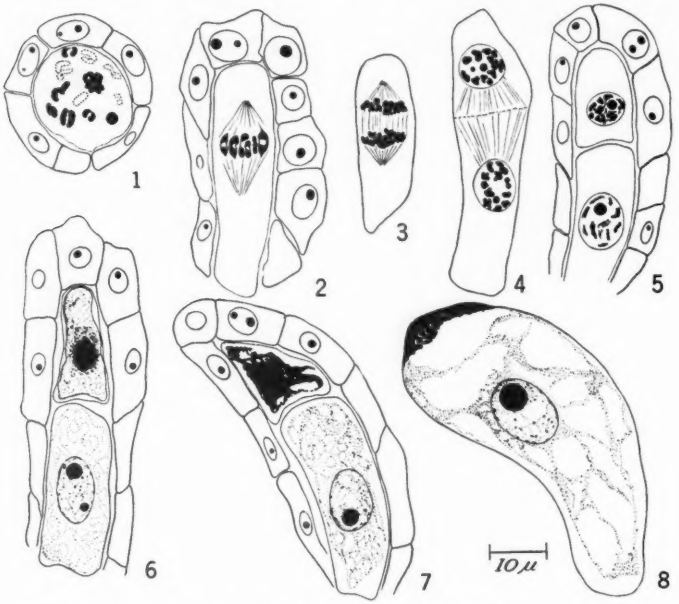
Megasporogenesis

The megaspore mother cell which differentiates from the subepidermal cells of the nucellus is easily recognized at an early stage in the development of the ovule by its larger size and by its affinity for the stain. During prophase of the first meiotic division the size of this cell and of its nucleus increases considerably. The typical stages of early prophase were seen. Beginning with the diakinesis stage, a detailed study was made of the meiotic divisions of the megaspore mother cell.

Figure 1 shows the 12 bivalent pairs of chromosomes lying within the nuclear membrane. Figure 2 shows a later stage after the nuclear membrane has disappeared and the spindle has formed. Bivalent chromosomes lie in the equatorial plane. Chromosomes then pass to the poles during anaphase I (fig. 3). Telophase I is recognized by the formation of a nuclear membrane around each of the two groups of 12 split chromosomes (fig. 4). A cell plate begins to form in the middle of the spindle, as shown in figure 4, and apparently develops a cell wall which separates the two daughter nuclei as shown in figure 5. A comparison with corresponding stages of development in our standard Line 1 *Datura* shows that the first meiotic division in dyad plants does not differ apparently from that found in normal individuals. There is, however, a distinct difference in the stages following the first division. The second meiotic division is never found in dyad plants, except in occasional cells to be discussed later. Apparently the daughter nuclei formed as a result of the first meiotic division go into a resting stage after a gradual telophase transformation (fig. 5). This stage persists during the interval when the second division ordinarily takes place. As a result of the failure of the second division only two megaspores (dyad) are formed instead of the usual four (tetrad). The megaspore that lies at the micropylar end is not viable. Its nucleus and cytoplasm gradually disintegrate (figs. 6, 7), while the cell at the chalazal end, together with its nucleus, enlarges greatly (fig. 8). By further divisions the megaspore thus formed gives rise to the female gametophyte.

FEMALE GAMETOGENESIS

In agreement with GUIGNARD (6), who described the development of the female gametophyte in *Datura laevis* (an inermis variety of *D. stramonium*), we find that the megaspore nucleus undergoes three successive mitotic divisions, resulting in the formation of an embryo

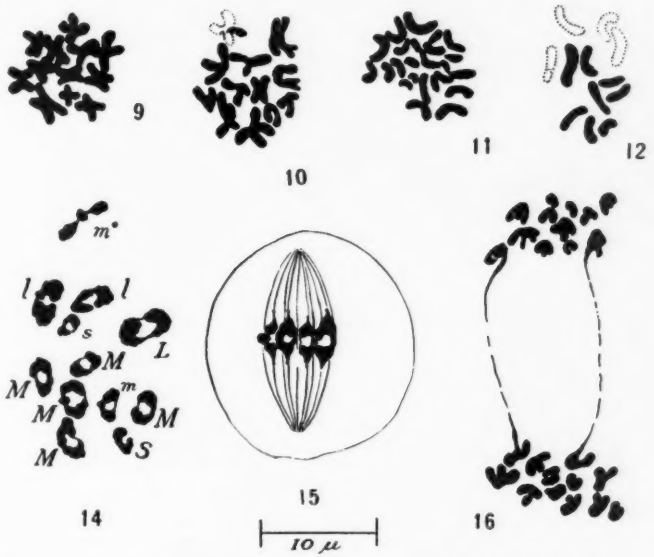


FIGS. 1–8.—Megasporogenesis in dyad plants of *Datura stramonium*. In figs. 3, 4 and 8 the cells of the nucellus are not shown: Fig. 1, 12 bivalents at diakinesis in megaspore mother cell. Fig. 2, metaphase I, six bivalents omitted. Fig. 3, anaphase I. Fig. 4, telophase I, two daughter nuclei each with 12 chromosomes within a nuclear membrane; formation of cell plate. Fig. 5, two megaspores (dyad), the lower with chromosomes indistinct in outline. Fig. 6, two megaspores; nucleus and cytoplasm of upper cell disintegrating; lower cell with resting nucleus. Fig. 7, later stage. Fig. 8, mature megaspore. ×850.

sac which contains eight nuclei. In dyad plants the first division is the most interesting, since it furnishes evidence in support of the conclusion that the second meiotic division is lacking. Figures 9 and 10 show the chromosomes of the megaspore in the dyad plant at the metaphase stage of this first meiotic division. Twelve *pairs* of chro-

mosomes are present instead of the 12 *single* chromosomes seen in the megaspore of normal plants shown in figure 12. The gametophyte of dyad plants thus starts out with 2n instead of with 1n chromosomes.

It is known, both genetically and cytologically, that at the end of the first meiotic division in normal plants each chromosome is



FIGS. 9–12, 14–16.—Figs. 9–11, mitosis in megaspores of dyad plants. Fig. 12, mitosis in megaspore of normal plant. Figs. 14–16, microsporogenesis in dyad plants: Fig. 9, metaphase of first mitotic division in megaspore showing 24 chromosomes lying in pairs. Fig. 10, similar stage with two chromosomes split. Fig. 11, metaphase of third mitotic division in megaspore showing 24 chromosomes. Fig. 12, metaphase of first mitotic division in normal megaspore showing 12 single chromosomes. Fig. 14, 12 bivalents at metaphase I in pollen mother cell. Fig. 15, metaphase I, eight bivalents omitted. Fig. 16, anaphase I, split chromosomes at each pole. ×1700.

really double, the two chromatids being separated from each other during the anaphase stage of the second meiotic division. Since this second meiotic division is lacking in dyad plants, these double chromosomes have had no opportunity to separate into two groups. Twelve *double* chromosomes therefore are what one would expect at

the metaphase of the first division of the megaspore. In figure 10 some of the chromosomes show a secondary splitting in anticipation of the second division of the megaspore.

At the metaphase stage of the second and third divisions, each nucleus has 24 single chromosomes. Figure 11 shows the chromosomes of one of the four nuclei in the third division. Apparently the paired condition of chromosomes seen at metaphase of the first mitotic division in the megaspore does not persist into later divisions.

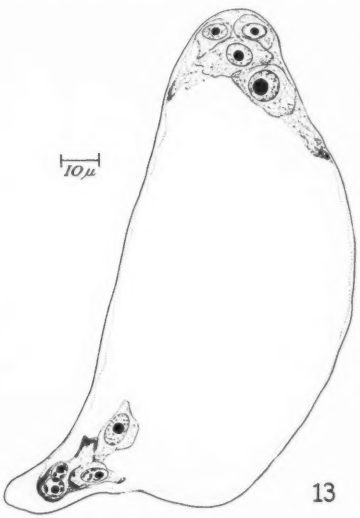


FIG. 13.—Female gametophyte in dyad plant shortly before fertilization. Egg cell, two synergids, and one polar nucleus at micropylar end of embryo sac; three disintegrating antipodal cells and one polar nucleus at chalazal end of embryo sac. ×550.

After the third division eight nuclei are formed, each containing 2n chromosomes. Cell membranes develop around six of these nuclei. The mature embryo sac contains at the micropylar end one egg cell and two synergids; the chalazal end has three antipodal cells which disintegrate very early. The two polar nuclei usually fuse near the egg cell. Figure 13 shows the beginning of disintegration of antipodal cells at an early stage when the polar nuclei are just beginning to move toward one another. The mature embryo sac of dyad plants is thus similar in structure to the embryo sac in a normal plant, except for the fact that each nucleus contains 24 instead of 12 chromosomes.

MICROSPOROGENESIS

The processes observed in pollen mother cells during microsporogenesis in dyad plants are essentially the same as those found in megasporogenesis. We have (a) an apparently normal first meiotic

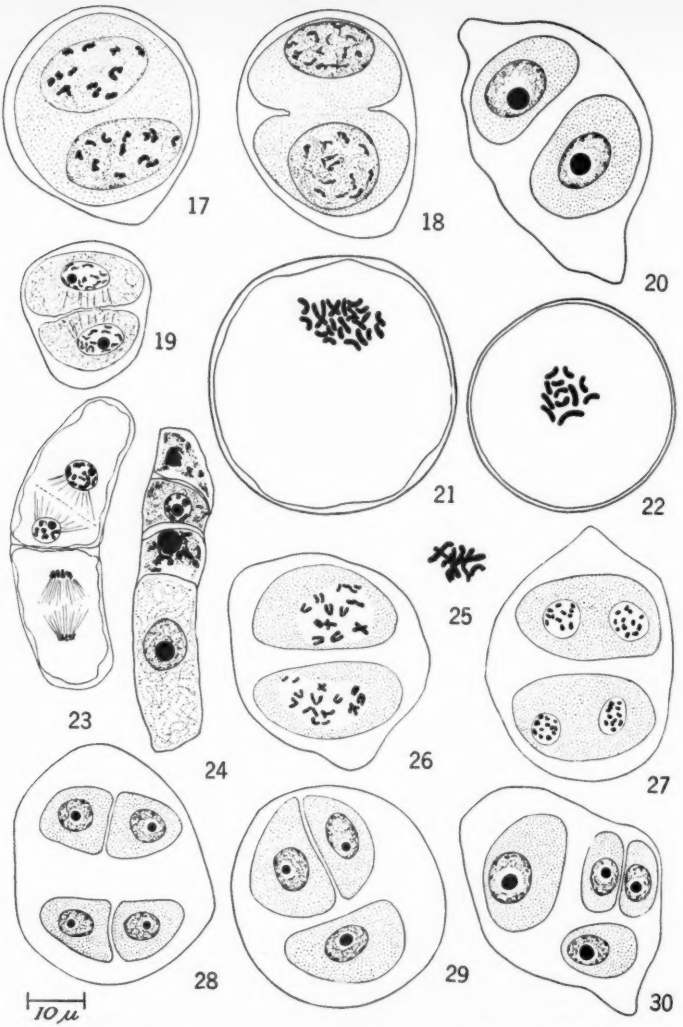
division; (b) a failure of the second meiotic division; (c) the formation of two microspores (dyads), instead of four (tetrads); and (d) the presence of 24 chromosomes in pollen grains. These conclusions, which were derived from a detailed study of microsporogenesis, confirm the earlier observations made by HOROWITZ.

Figure 14 shows metaphase of the first meiotic division in the pollen mother cell with 12 apparently normal bivalents in the equatorial plane. Figure 15 is a side view of the same stage and shows the spindle. After the separation of the bivalents in anaphase I, 12 split chromosomes are found at each pole of the spindle, as is shown in figure 16. At an early stage of telophase I the number of chromosomes can be counted (fig. 17). A nuclear membrane is formed around each group. As the telophasic transformation proceeds, the chromosomes in nuclei become slender, irregular, and indistinct in outline (fig. 18). In normal *Datura* species, interkinesis is of brief duration. The chromosomes are never completely lost from view. In dyad plants, however, "interkinesis" proceeds until a real resting stage is reached.

In *Datura* cytokinesis is simultaneous, that is, there is no division of the cytoplasm until after telophase II. No cell plates are formed on the spindles between the daughter nuclei. Cytokinesis begins with the formation of furrows which move in from the edges of the cell to divide the cytoplasm into four portions. Thus a tetrad of young microspores is formed within the pollen mother cell. In dyad plants, cytokinesis begins soon after the first telophase and before the nuclei have reached the resting stage. A furrow moves (fig. 18) in from the edges of the cell and bisects the pollen mother cell into a dyad of microspores. Figure 19 shows an advanced stage of cytokinesis although a few achromatic fibers are still present. By the time the nuclei have reached the resting stage, a thick cell wall has been formed between them (fig. 20). Later the two microspores thus formed slip out of the membrane of the pollen mother cell.

MALE GAMETOPHYTE

The development of the male gametophyte in dyad plants is quite normal, except for the number of chromosomes involved. As in nor-



FIGS. 17–30.—Figs. 17–20, microsporogenesis in dyad plants (continued). Fig. 21, mitosis in pollen grain in dyad plant. Fig. 22, same in normal plant. Figs. 23–25, exceptional cases in megasporogenesis and female gametogenesis in dyad plants. Figs. 26–30, same in microsporogenesis in dyad plants: Fig. 17, telophase I in pollen mother cell, two daughter nuclei each with 12 chromosomes within nuclear membrane. Fig. 18, further development; chromosomes indistinct in outline; beginning of cytokinesis by furrow at edge of cell. Fig. 19, advanced cytokinesis; pollen mother cell bisected into a dyad of microspores; few achromatic fibers still present. Fig. 20, microspores in dyad, with resting nuclei. Fig. 21, metaphase stage of mitotic division in pollen grain of dyad plant showing 24 chromosomes. Fig. 22, metaphase stage of mitotic division in pollen grain of normal plant showing 12 chromosomes. Fig. 23, completed second meiotic division. Fig. 24, four megaspores, three of which degenerate; nucleus of lower megaspore at resting stage. Fig. 25, female gametogenesis; metaphase of third mitotic division of nucleus showing 12 chromosomes. Fig. 26, second metaphase in a dyad. Fig. 27, second telophase. Fig. 28, tetrad of microspores. Fig. 29, triad of microspores. Fig. 30, tetrad of microspores of unequal size. ×850.

mal *Datura* species, each young pollen grain has a single large nucleus in the middle of the cell and a dense cytoplasm. A thick exine develops gradually. Just before the pollen grain becomes opaque, owing to the development of starch, the nucleus, which now lies close to one edge of the cell, goes through a mitotic division. Figure 21 shows the metaphase stage of this division. Since there was no second meiotic division, each microspore of a dyad plant has 12 double chromosomes; that is, 24 chromosomes instead of the usual 12 single ones of normal plants shown in figure 22. In figure 21 there is a suggestion of pairing of sister chromosomes like those shown in the first meiotic division of the megaspore (figs. 8, 10). Around one of the daughter nuclei a membrane cuts out the generative cell, separating it from the other nucleus. This latter nucleus with the rest of the pollen grain becomes the vegetative cell of the male gametophyte.

Exceptional cases

It should be mentioned that a few megaspore mother cells develop in the same way as those of normal plants, in that both the first and the second meiotic divisions take place. (Figure 23 shows such a second division in a dyad plant.) As a result, four megaspores are formed. Three of these disintegrate (fig. 24) and the nucleus of the megaspore which remains viable has 12 single chromosomes, as is the case of normal *Datura* (fig. 12). The succeeding nuclei of the gametophyte will therefore have only 12 chromosomes. Figure 25, for example, shows the third mitotic division of one of these nuclei with 12 chromosomes from a dyad plant. The number of megaspore mother cells which showed second divisions varied considerably. In most of the buds examined no such cases were found. In other cases there must have been approximately 1 to 5 per cent of second divisions, judging from the number of second divisions actually observed or calculated from the presence of four megaspores.

The same exceptional occurrence of the second meiotic division has been observed during microsporogenesis. This division takes place either soon after telophase I, as in normal plants, or later, after a thick wall has separated the two dyads. As a result, four microspores are formed, each with 12 chromosomes as in normal plants

(figs. 26–28). Rarely one only of the two dyad cells undergoes a division, thus giving rise to three microspores (fig. 29), one with 24 and two with 12 chromosomes. In two cases a further, supernumerary division has been observed in one of these two microspores, which had resulted from a second division. Two microspores of half normal size (fig. 30) are thus formed.

The cytological observations can be checked in two ways: by the breeding behavior and by the sizes of the pollen grains. Dyad plants can be used as females, in crosses to normals, although the number of seeds set is much below average. The offspring are chiefly diploids which show both paternal and maternal characters, indicating that they have been derived from 1n egg cells, which are those resulting from the exceptional cases of second meiotic divisions. In a few cases the cross of a dyad by a normal gave a triploid which must have come from the union of a 2n egg with a normal 1n sperm. We have also had a dyad seedling come from pollinating a dyad plant with normal pollen. This we have interpreted as due to parthenogenesis of a 2n egg.

Pollen of dyad plants has not been successfully used in crosses with normals, presumably because the tubes from grains with 2n chromosomes burst in the styles of normal diploids (5). Such 2n grains have approximately twice the volume of the 1n grains found in normal diploids, and are easily distinguished from the latter. From the occasional admixture of 1n grains among otherwise 2n pollen, it can be concluded that a second division takes place in about 1 to 5 per cent of the pollen mother cells of dyad plants. Sometimes no 1n pollen grains can be found in an anther, and rarely has the number reached more than 50 per cent.

Discussion

As is known from the literature, various irregularities which occur during meiosis may result in the doubling of the entire chromosome complement. Only two such cases need be mentioned here: (1) BEADLE (2) has reported the failure of cytokinesis in maize after the first and second meiotic divisions or after only the second division. In such cases pollen grains with 4n or with 2n chromosomes are formed. (2) Another abnormality in sporogenesis, "the semi-hetero-

typic division," is described by ROSENBERG (7) in parthenogenetic *Euhieracium*. The first meiotic division is interrupted at various stages by a premature homoeotypic division. The entire spindle is surrounded by a nuclear membrane. A single "restitution nucleus" is formed which has the diploid number of split chromosomes. Since this homoeotypic division is the only one which is completed, dyad microspores with 2n chromosomes are formed. Similar conditions have been found by other investigators. In the gene "dyad" which has been described here, it is the failure of the second meiotic division which leads to the formation of a diploid number of chromosomes in the gametes. The exact mechanism by which the gene suppresses the second division is not known, but the effect appears to be brought about through a premature cell wall formation which anticipates the second division, or, perhaps more likely, through a great extension of the telophase stage which in normal plants is of brief duration. Whatever may be the immediate effect of the gene by means of which it brings about dyad formation, its action is influenced by environmental factors, as shown by the exceptional cases of tetrad formation.

Summary

1. Dyad formation in sporogenesis in *Datura*, owing to a recessive gene, was found among the F_2 offspring from radium-treated pollen.

2. During megasporogenesis the first meiotic division is present, but the second meiotic division is regularly absent. As a result only two megaspores (dyads) are formed.

3. One of the megaspores disintegrates; the other undergoes three successive mitotic divisions. Its nucleus contains 12 pairs of chromosomes instead of 12 single chromosomes.

4. An embryo sac is formed containing eight nuclei, each with 24 chromosomes.

5. During microsporogenesis the same meiotic abnormality is found in the pollen mother cell. There is a failure of the second division, and after the first division a thick cell wall develops between the two nuclei, forming a dyad of microspores.

6. Each microspore, containing 12 pairs of chromosomes, develops into the male gametophyte.

7. A small percentage (about 1 to 5 per cent) of megasporocytes

and microsporocytes in dyad plants develops normally. Since both meiotic divisions are present, a "tetrad" of microspores instead of a "dyad" is formed. Each gamete that results from these exceptional cases contains 12 single chromosomes.

8. The formation of 2n and 3n normal offspring from the cross dyad×normal confirms the occurrence of both 1n and 2n egg cells. Dyad 2n offspring have come from parthenogenesis of a 2n dyad egg.

9. The occurrence of large and normal sized pollen grains in dyad plants confirms the occurrence of both 1n and 2n sperms.

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CULTURAL CHARACTERS AND PAIRING REACTIONS OF MONOSPOROUS MYCELIA AND DEVELOPMENT OF THE FRUIT BODY OF PHOLIOTA (FLAMMULA) POLYCHROA[1]

ALEXANDER H. SMITH AND HAROLD J. BRODIE

(WITH THIRTY FIGURES)

Introduction

Pholiota polychroa (Berk.) Smith & Brodie was originally described from material collected at Waynesville, Ohio, but it has been found rather frequently in the region about the Great Lakes and along the eastern seacoast from Virginia northward, usually on partially decayed fallen trunks or branches of oak and maple. In the vicinity of Ann Arbor this fungus seems to grow most frequently upon oak, but may also be found on maple. In the northern peninsula of Michigan it has been found growing on hemlock as well as on oak. It would be desirable to make a comparative study of collections of *Pholiota polychroa* from both coniferous and deciduous woods, with a view to ascertaining whether or not two different strains exist, but the writers have not had the opportunity of doing this. All the material which served for the present study was collected from oak logs in the vicinity of Ann Arbor.

Investigation

GERMINATION OF BASIDIOSPORES

From a carpophore of *Pholiota polychroa* collected November, 1933, a spore deposit was obtained. The spores are rich dark brown in color and on the average measure 6–7.5×3–4 μ. They germinated readily 12 hours after they had been sown on Kauffman's synthetic

[1] Papers from the Department of Botany and the Herbarium of the University of Michigan, no. 474.

As a result of their study of this fungus, as reported herewith, the writers propose to place it in the genus *Pholiota*.

agar (4), on malt extract agar,[2] or in an aqueous solution of malt extract.

In order to study the behavior of nuclei during germination, spores were germinated in malt extract solution, were fixed and stained in the following manner. About 18 hours after the spores had been placed in the nutrient solution, the greater part of the liquid was decanted from the spores, which had settled to the bottom of the container. A quantity of weak Flemming's solution was then added to the residue and the spores allowed to stand for about one hour in the fixative. A thin layer of Szombathy's fixative was smeared over the surface of a glass slide and a drop of a 4 per cent solution of formalin was placed in the center of the slide. To this drop was added a drop of the solution containing the germinated spores. The slide was allowed to stand until the liquid had evaporated; it was then washed for 15 minutes in distilled water, bleached, and stained as desired. The use of Haidenhain's haematoxylin alone gave fairly good results. Particularly brilliant staining of the nuclei was obtained by the use of safranin.

The basidiospore possesses an apical pore through which the protoplasm of the spore emerges, forming a vesicle (figs. 1–4). That the germ pore is actually apical in position is demonstrated by the fact that in sections of the hymenium, spores still attached to the sterigmata of the basidia were seen to be provided with germ pores at their apical ends (fig. 20).

Two nuclei are usually present in the mature spore (figs. 1, 20). Upon germination, these nuclei migrate into the vesicle. Figure 2 shows one nucleus in the spore and one in the vesicle, while in figures 3 and 4 there are two nuclei in the vesicle.

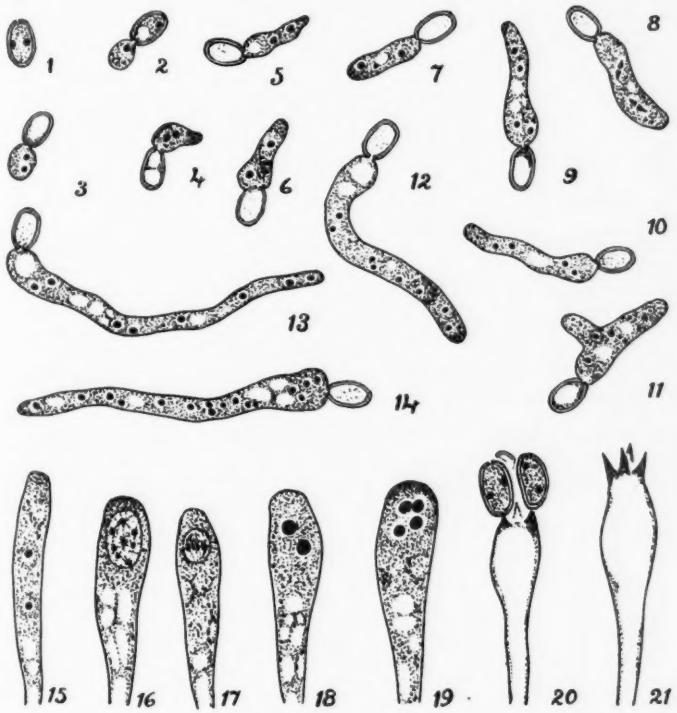
As the germ tube begins to develop from the vesicle (figs. 5, 6, 7), the two nuclei divide simultaneously (fig. 8), giving rise to four (figs. 9, 10, 11); and as development proceeds these give rise to eight, sixteen, and more nuclei (figs. 12, 13, 14). That the number of nuclei observed was either two, four, eight, or sixteen indicates that they divide simultaneously, a conclusion which is further upheld by their disposition in pairs (fig. 12). No attempt was made to follow the

[2] Prepared according to the following formula: agar 15 gm., malt extract (Merck and Co.) 15 gm., distilled water 1 liter.

multiplication of nuclei beyond the point where there were sixteen present. At this stage the young mycelium is still aseptate.

Cultural studies and analysis of sexuality

The mycelium which develops from a single basidiospore of *Pholiota polychroa* is haploid. It is composed of rather fine hyphae, the



Figs. 1–21.—Figs. 1–14, stages in germination of basidiospores. ×900. Figs. 15–21, nuclear history and basidiospore formation: fig. 15, primary nuclei before fusion; figs. 16, 17, fusion nuclei; fig. 18, two nuclei produced by first division of fusion nucleus; fig. 19, four nuclei in basidium prior to spore formation; fig. 20, mature basidium showing two spores only (note the two nuclei in each basidiospore); fig. 21, empty basidium showing no nuclei remaining after spores have been shed. ×1130.

average width of which is about 4 μ. The mycelium tends to lie flat on the agar substratum and does not present a fluffy appearance.

Oidia are produced in abundance on the haploid mycelium by the fragmentation of special spirally coiled hyphae. The oidia were found to germinate under suitable conditions.

Thirty single-spore mycelia were obtained from the spore deposit. They were grown on malt extract agar in tubes. Two months after they had been isolated, the mycelia were paired in all possible combinations on agar slants (435 pairings). Two weeks later these pairs were examined. In some of the tubes the two mycelia had intergrown without any apparent reaction having taken place between them. In other tubes the two mycelia had given rise to a diploid mycelium.

The diploid mycelium develops in the region of contact of the two haplophytes and then proceeds to grow over them. It differs from the haploid mycelium in producing a dense aerial growth. The development of diploid mycelium in a restricted area between the compatible haplophytes has been discussed by VANDENDRIES (**8**) in connection with his studies of *Trametes suaveolens;* and unpublished studies by Mrs. C. A. ARNOLD in this laboratory have demonstrated that a restricted diploidization also exists in certain species of *Collybia*. The difference in appearance between the haploid and diploid mycelium is illustrated by figure 22.

The appearance of the diploid mycelium developed between the two compatible haplophytes in culture tubes was so distinctive that macroscopic examination alone indicated which pairs of haplophytes were sexually compatible and which incompatible. The pairings of mycelia nos. 1 and 2 with each of the other 28 mycelia were examined also microscopically. In every instance clamp connections were found to be present on mycelia which had been marked as showing a positive reaction, and no clamp connections were found on mycelia which showed a negative reaction.

The behavior of the 30 single-spore mycelia was exceedingly regular. In none of the cultures examined microscopically were there any "false clamp connections" (**9**). In all the pairings, the appearance of diploid mycelium between any two haplophytes was always in accordance with the genetic formulae which could be ascribed to them from the results of their reactions with the other mycelia of the series. In none of the pairings was there any sign of the "barrage" phenomenon recently described by VANDENDRIES and BRODIE (**9**).

The results of the pairing experiment are incorporated in table I. In this table a plus sign indicates the presence of diploid mycelium; a minus sign, its absence.[3] It may be concluded that *P. polychroa* is heterothallic and tetrapolar.

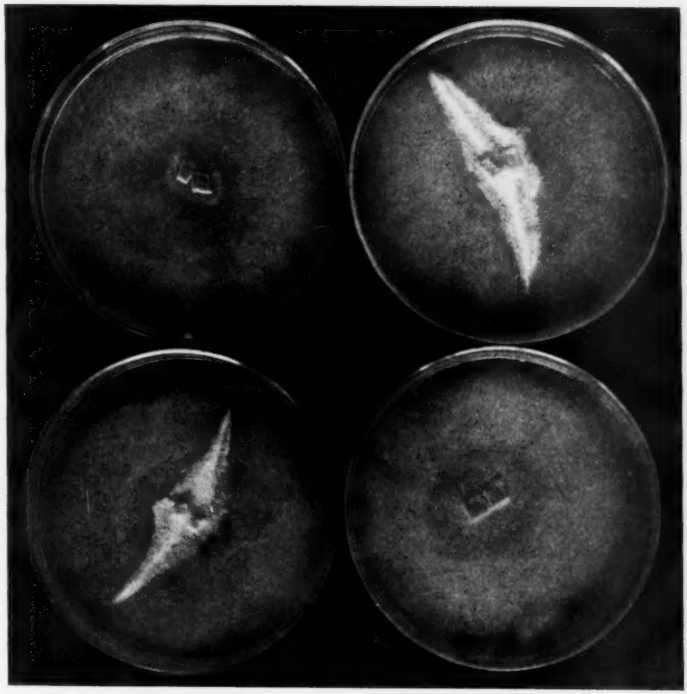


FIG. 22.—Haploid mycelia paired on agar plates showing localized development of diploid mycelium. Haploid mycelium grows compactly along surface of agar whereas diploid mycelium forms fluffy white aerial growth. ×0.5.

Attempts were made to induce sporophore formation on diploid mycelium. Three flasks each of oatmeal, cornmeal, 2.5 per cent malt extract agar, and ETTER's medium (1) were prepared. In the last, finely ground sawdust (chiefly of oak and maple) was used. The

[3] Cultures 1, 2, 3, 4, 5, 6, 8, and 9 have been deposited in the Centraal Bureau voor Schimmelcultures at Baarn, Holland.

flasks were inoculated on December 2, 1931, and were placed in a darkened room maintained at a temperature of about 20° C.

The mycelia grew luxuriantly but no fruit bodies developed. The cultures eventually became dry. At the end of January, the mycelia

TABLE I

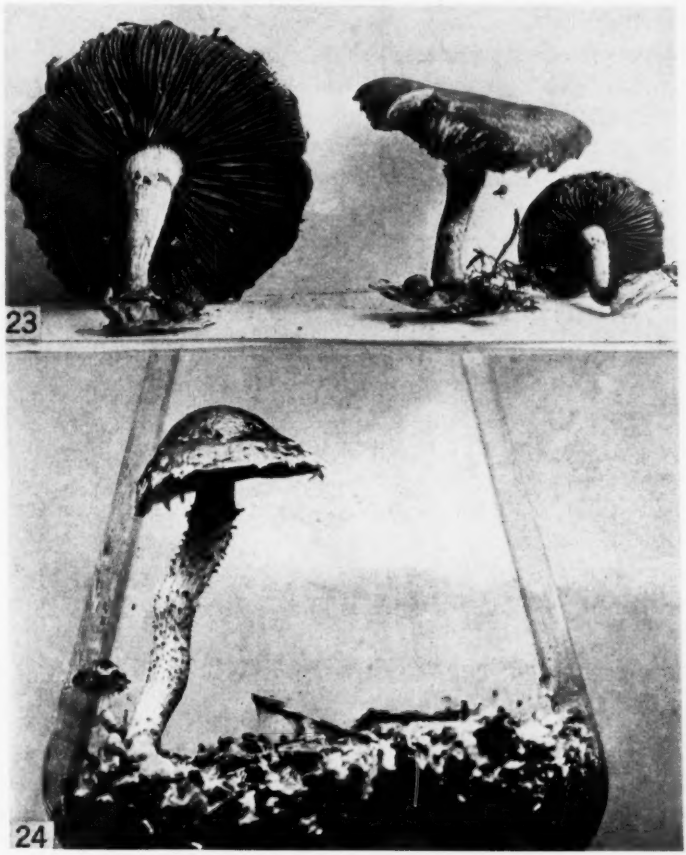
RESULTS OF PAIRING IN ALL POSSIBLE WAYS THIRTY SINGLE-SPORE MYCELIA OF PHOLIOTA POLYCHROA

		AB						ab							Ab						aB						
		8	9	15	21	23	29	30	1	2	10	11	12	16	18	24	27	5	6	17	19	22	26	3	4	7	13	14	20	25	28
AB	8		−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	9	−		−	−	−	−	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	15	−	−		−	−	−	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	21	−	−	−		−	−	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	23	−	−	−	−		−	−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	29	−	−	−	−	−		−	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	30	−	−	−	−	−	−		+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
ab	1	+	+	+	+	+	+	+		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	2	+	+	+	+	+	+	+	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	10	+	+	+	+	+	+	+	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	11	+	+	+	+	+	+	+	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	12	+	+	+	+	+	+	+	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	16	+	+	+	+	+	+	+	−	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	18	+	+	+	+	+	+	+	−	−	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	24	+	+	+	+	+	+	+	−	−	−	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	27	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−		−	−	−	−	−	−	−	−	−	−	−	−	−	−
Ab	5	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−	−	−	+	+	+	+	+	+	+	+
	6	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−	−	+	+	+	+	+	+	+	+
	17	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−	+	+	+	+	+	+	+	+
	19	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	+	+	+	+	+	+	+	+
	22	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	+	+	+	+	+	+	+	+
	26	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		+	+	+	+	+	+	+	+
aB	3	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+		−	−	−	−	−	−	−
	4	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−		−	−	−	−	−	−
	7	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−		−	−	−	−	−
	13	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−		−	−	−	−
	14	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−		−	−	−
	20	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−		−	−
	25	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−	−		−
	28	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−	−	−	

on ETTER'S medium were moistened with a sterile solution of 2.5 per cent malt extract and by the end of February a few malformed rudiments of fruit bodies were found in one culture. Again malt extract solution was added, but no further development of the rudiments took place.

On March 13 the three cultures on ETTER'S medium were placed on a table near a window facing east, but not in direct sunlight, and

again moistened with malt extract solution. By April 1, button stages of sporophores had developed on the mycelium in one flask, and on April 8 a fruit body 4 cm. broad had matured. The sporophore was in every respect typical (fig. 24), having all the characters ascribed to the species by KAUFFMAN (3).



FIGS. 23, 24.—Fig. 23, fruit bodies collected and photographed by E. B. MAINS near Munising, Mich., 1932; fig. 24, fruit body which developed in culture on ETTER'S medium, April 8, 1932. ×1.

The cultures were kept moist and eventually three crops of sporophores were obtained. The second developed about four weeks after the first and the last crop three weeks after the second. The spores from the fruit bodies produced last were only about 25 per cent germinable, although spores from the first crop of fruit bodies were nearly 100 per cent germinable. Only one of the three cultures on ETTER'S medium produced any fruit bodies.

Experiments made later under more carefully regulated conditions failed to provide more sporophores, and it has not been found possible up to the present time to manipulate the conditions of culture so that the fruit bodies of *P. polychroa* can be produced when desired.

DEVELOPMENT OF FRUIT BODY

The material used for the developmental studies and the carpophore which provided the foregoing cultures were both obtained from the same locality. Material was killed and fixed in Allen's modification of Bouin's solution and was imbedded in paraffin in the usual manner.

STEM PRIMORDIUM.—The youngest fruit body sectioned was somewhat egg-shaped and its stem primordium was situated apically. Figure 25 represents a stage of development in which the stem fundament has just reached the upper surface of the basidiocarp primordium and begun the formation of the stipe. The writers believe, as SAWYER (7) and others have pointed out, that differentiation of the stem fundament actually begins near the base of the small, compact, subglobose mass of hyphae forming the fruit body primordium. At the stage of development shown in figure 25, the broad base is made up of compactly interwoven hyphae which measure 4–6 μ in diameter and which stain lightly but uniformly. Just above this area, in the oval part of the young fruit body, the hyphae stain more intensely and measure only 2–3 μ in diameter. The ground tissue located next to this dark area (which is the stem fundament) is also made up of narrow hyphae, but their arrangement is looser than that of the hyphae in the central layer.

DIFFERENTIATION OF HYMENOPHORE AND PILEUS PRIMORDIA.—The earliest stage found illustrating the development of the hymenophore primordium is shown in figure 26. Here a narrow band of

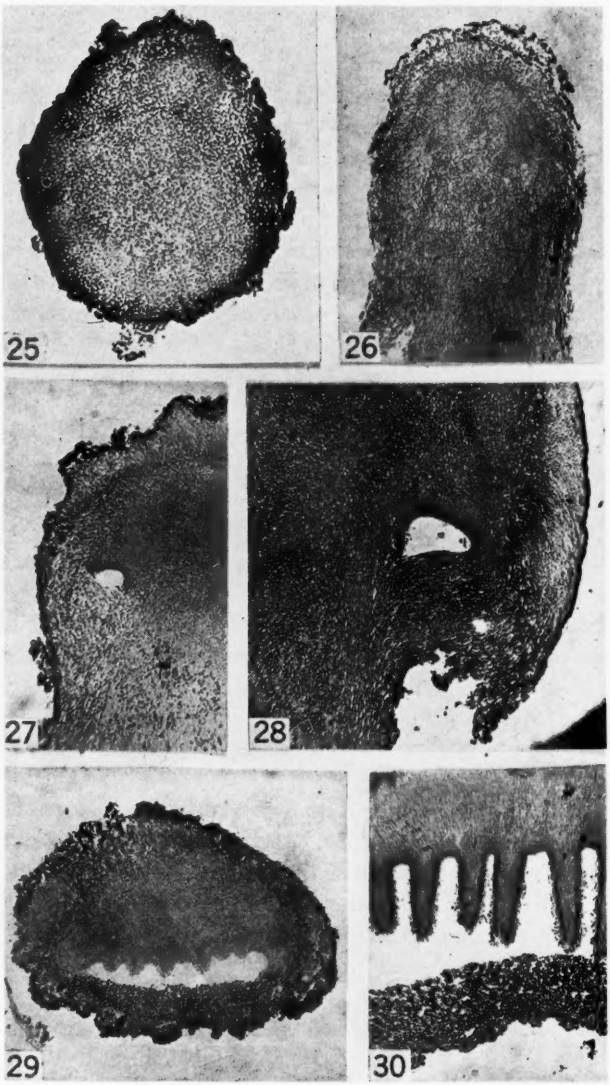
darkly staining hyphae can be seen, the ends of which point more or less toward the base of the basidiocarp. The tissue immediately beneath these hyphae stains lightly and is of very loose structure. At this stage there is no clearly defined palisade layer nor gill cavity.

A rather broad zone of narrow, deeply staining hyphae differentiates the pileus from the universal veil tissue, which stains lightly. The hymenophore and pileus primordia are separated by a narrow band of hyphae. It has been impossible to determine with certainty which of these two primordia is differentiated first. Apparently they originate at about the same time.

In figure 27 the blematogen layer can be recognized. This is very distinct in figure 28, which illustrates a young sporophore 3 mm. in width, because of the difference in the capacities of the two tissues for holding the stain. Eventually the greater part of the universal veil tissue becomes gelatinized, and as the cap expands, the tissue becomes thinner and more amorphous. In carpophores with well developed blematogenous tissue only the lower portion gelatinizes; and as the pileus expands, floccose patches are left scattered over the surface of the pileus, particularly on the margin. Both the partial and the universal veil contribute, however, to the formation of the floccose scales on the margin. In some fruit bodies only marginal scales are found, either because the entire blematogenous layer gelatinizes or because the small scales are removed mechanically during the expansion of the fruit body.

ORIGIN OF GILL CAVITY, LAMELLAE, AND PARTIAL VEIL.—As the hyphae grow downward to form the palisade layer (fig. 27), the ground tissue beneath becomes torn. At first the cavity thus produced is crossed by occasional hyphae; later it enlarges and is clearly defined (fig. 28). The palisade layer is rather uneven at first, but soon becomes very compact near the stipe.

The lamellae originate as obtuse folds near the stipe, and grow downward into the gill cavity as well as outward toward the margin of the pileus. The gill primordia can be distinguished (fig. 29) as areas of fine, darkly staining hyphae which push down on the palisade layer, eventually giving rise to definite folds which are the young gills. The growth of the gills continues and soon the narrow lamellae are easily discernible in the gill cavity (fig. 30). Growth



FIGS. 25–30.—Fig. 25, young basidiocarp showing stem primordium, ×36; fig. 26, same showing differentiation of pileus and hymenophore primordia, ×34; fig. 27, development of gill cavity and further differentiation of universal veil, ×20; fig. 28, gelatinization of universal veil (note that gelatinization progresses only a short distance beyond margin of pileus), ×48; fig. 29, development of gill primordia and formation of young gills, ×50; fig. 30, young gills and veil in partly expanded fruit body, ×26.

may continue until the edges of the gills come into contact with or are even pushed into the veil tissue at the base of the cavity.

Both the partial and the universal veil are distinguishable at their points of conjunction with the pileus but become indistinguishable as they merge into the membranous veil. The gelatinization of the universal veil (fig. 28) progresses only slightly beyond the margin of the pileus. As the pileus expands and the stipe elongates, the veils are ruptured, leaving innate scales on the stipe and soft fibrillose or membranous patches scattered on the pileus, or appendiculate along the margin.

The cytology of basidiospore formation follows the usual course. Two primary nuclei (fig. 15) fuse and two divisions of the fusion nucleus follow (figs. 16–19). One nucleus migrates into each basidiospore and there divides so that the mature spore contains two sister nuclei (fig. 20). The writers could find no indication of any nuclei left behind in the basidium after the spores had formed and matured (figs. 20, 21) and are therefore certain that the binucleate condition of the mature basidiospore results from the division of a single nucleus.

The development of this fungus agrees in all essentials with the development of species of *Pholiota* studied by SAWYER (**7**). The development of the stem, pileus, and hymenophore are not of great importance because they are similar in most endogenously developing agarics except those of the *Amanita* type. In *Pholiota polychroa* the morphology and mode of development of the universal veil and the partial veil are practically identical with the development of these parts in *P. flammans* and *P. adiposa* (**7**). Inasmuch as little is known concerning the development of the species of *Flammula*, it is at present impossible to make a detailed comparison between the two genera *Flammula* and *Pholiota* from the standpoint of the development of the fruit body, or to attempt to establish generic limits on the basis of developmental studies. Undoubtedly such a study must be made before all the border-line species can be properly classified according to their natural relationships.

TAXONOMIC POSITION OF PHOLIOTA POLYCHROA

This fungus has at different times been referred to *Hypholoma*, *Pholiota*, and *Flammula*. PECK, who placed the species in the sub-

genus *Hypholoma* under *Agaricus* (6), later removed it to *Pholiota* (*P. ornella*), apparently after he had carefully studied the color of the spore print. The spores are of a rich dark brown color but not purple-brown as in *Hypholoma*. Whether the species under consideration more properly belongs in *Pholiota* or in *Flammula* is a matter difficult to decide, because the distinction between the two genera rests largely upon the character of the veil; the degree of its differentiation rather than its presence or absence has been considered of importance in the past.

Concerning the relationship of the two genera, FRIES (2) says of *Pholiota*, "sine distinctis limitibus transit in Flammulas"; and OVERHOLTS (5), "At some points the genus grades into *Flammula* due to the early disappearance of the partial veil or the annulus, and in *Flammula* the veil fragments may at times persist as an incomplete annulus." Such statements indicate that in placing collections in either genus one is often guided more by the opinions of previous workers than by the characters of the fungi in hand. The prominence of the well developed veil in the ontogeny of the fruit body of *Pholiota polychroa* indicates a closer relationship to certain species of *Pholiota* than to other species of *Flammula*.

Macroscopically *P. polychroa* is a typical border-line species. It is often found to possess the soft floccose scales on the pileus and along the margin and a definite subapical annular zone around the stipe. The stipe may also be covered with soft innate scales for some distance below the annular zone. These characters indicate a relationship to *Pholiota flammans* and *P. adiposa*.

Under relatively dry conditions, sporophores may be nearly destitute of a veil and the pileus may be entirely glabrous. Such specimens, which are frequently collected, no doubt account for the species being retained in *Flammula*. The fruit bodies shown in figure 23 illustrate this condition, but even here the annular zone is clearly visible.

From the preceding account it is apparent that there is not sufficient reason for keeping the fungus in the genus *Flammula*. The species shows a striking relationship to *Pholiota* in the manner of its development and in its stature, and it is distinguished from *Flammula* by possessing a veil which is well developed. The writers be-

lieve the fungus is more properly classified as a *Pholiota*, and therefore propose the combination *Pholiota polychroa* (Berk.) comb. nov.

SYNONYMS

Agaricus polychrous Berk.—Lond. Jour. Bot. **6**:313. 1847.

Agaricus ornellus Pk.—Ann. Rep. New York State Mus. **34**:42. 1883.

Flammula polychroa Sacc.—Syll. Fung. **5**:824. 1887.

Pholiota appendiculata Pk.—Bull. New York State Mus. **94**:33. 1905.

Pholiota ornella Pk.—Bull. New York State Mus. **122**:151. 1908.

Gymnopilus polychrous (Berk.) Murr.—North Amer. Flora **10**: 204. 1917.

Summary

1. The basidiospores of *Pholiota polychroa* germinate readily on various media in 12–18 hours. A large germinal vesicle is formed first and the two nuclei present in the mature spore migrate into it before dividing. As the vesicle and germ tube develop, nuclear division takes place. In the early stages of the development of the haploid mycelium, the nuclei divide simultaneously and ceonocytic cells with two, four, eight, and sixteen nuclei are commonly found.

2. A pairing experiment using 30 monosporous mycelia showed this species to be heterothallic and tetrapolar. Diploidization takes place only along the line of contact between the two compatible haplophytes. The diploid mycelium grows slowly out over the haploid mycelium.

3. Typical sporophores producing germinable spores were obtained in pure culture, but it has not been possible as yet to manipulate the conditions of culture so that sporophores can be developed as desired.

4. It was found that the lamellae of the fruit body develop endogenously and that a pronounced gill cavity is formed. The manner of development and the type of veil produced indicate a close relationship to species of *Pholiota*, and the species is transferred from *Flammula* to *Pholiota*.

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SALIENT LINES OF STRUCTURAL SPECIALIZATION IN THE WOOD RAYS OF DICOTYLEDONS

DAVID A. KRIBS[1]

(WITH PLATE VIII AND ONE FIGURE)

Introduction

In recent years numerous attempts have been made to establish correlations between anatomical characters of the stem and morphological characters of the flowers, fruits, and leaves in a supposedly natural classification of the dicotyledons. The results obtained thus far are more or less contradictory. High correlations frequently occur in groups of closely related species and genera, whereas serious discrepancies arise in the case of large families and orders. If serious confusion is to be avoided, wood technologists should devote more attention to the study of the main lines of structural specialization in the evolutionary development of the dicotyledons as a whole.

BAILEY and his co-workers (**2, 3, 4, 6, 7, 8**) have shown that the salient lines of structural specialization in the cambium and tracheary elements of dicotyledons are so distinct and so closely correlated that they may be studied effectively by statistical methods. Their investigations demonstrate that the fusiform initials of the cambium and their derivatives are reduced in length as specialization increases. As the vessels of the secondary xylem become more and more highly differentiated, their segments gradually lose their resemblance to tracheids, and tend to become progressively wider and shorter. At the same time the structure and arrangement of the bordered pits in their lateral walls tend to be considerably modified. Furthermore, the surrounding tracheary elements tend to shorten and to take on a more fiber-like structure, their pits becoming vestigial by the gradual disappearance of the bordering areas of the secondary walls.

The writer has endeavored to trace major lines of specialization in the wood rays and wood parenchyma of dicotyledons. The present paper deals with the wood rays; the distribution of wood parenchyma will be discussed in a subsequent paper.

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Methods

Methods essentially similar to those of BAILEY and TUPPER (**4**) and FROST (**6**) were followed, that is, the statistical method was applied to the results of a detailed anatomical investigation of the woods in Professor BAILEY's collection. The collection is representative of the woods of the world and in 1929 it included 63 of the 76 orders and 137 of the 264 families listed by HUTCHINSON, approximately 800 genera and 2500 species being represented. Since that time numerous woods from various parts of the world have been added to the collection.

As FROST (**6**) has pointed out, "the statistical method is essentially a method for the study of the characteristics of a large group by taking individual samples, at random, from the group. Experience in agreement with theory has repeatedly shown that the accuracy of conclusions drawn from statistical results is proportional to the number of cases used in deriving the statistical constants. For this reason the number of individual woods measured and studied in these investigations is large in order to insure a fair representation of dicotyledonous woods in general."

The woods were divided into six groups upon the basis of the structure of their tracheary tissue, as follows: scalariform I, scalariform II, scalariform-porous, porous-oblique, porous-oblique and transverse, and porous-transverse. The ray types and wood parenchyma types were then studied in detail for each group separately. This procedure reveals certain diagnostic criteria which might otherwise be overlooked.

Discussion

Hitherto wood rays have been placed in the following categories: uniseriate, compound, aggregate, multiseriate, heterogeneous, and homogeneous. According to the latest publication by the Committee on Nomenclature, International Association of Wood Anatomists (**12**), three types of wood rays are recognized: *aggregate ray* (a group of small, narrow, xylem rays appearing to the unaided eye or at low magnification as a single large ray); *homogeneous ray* (a xylem ray composed of radially elongated cells); *heterogeneous ray* (a xylem ray composed of cells of different morphological types, typically with

the cells of the multiseriate part radially elongated and those of the uniseriate parts vertically elongated or square).

One would infer, from these definitions of the terms *homogeneous* and *heterogeneous*, that the Committee has restricted the use of these terms exclusively to the multiseriate ray. Concomitant *uniseriate* rays evidently have not been taken into consideration. This is especially important, not only from the standpoint of formulating keys to the identification of woods, but also because of its great assistance in elucidating a phylogenetic sequence. It is suggested that any subsequent terminology should include the uniseriate ray in association with the multiseriate, thus eliminating the present ambiguous application of the terms.

In correlating wood rays with the various types of vessel elements, certain specific ray combinations were conspicuously brought to the writer's attention: (1) wood rays may be divided into six distinct types; (2) in two distinct ray types, the cells which constitute the uniseriate rays differ in size and form from the cells of the multiseriate portion of the multiseriate rays; (3) conversely, in two other distinct ray types, the cells which constitute the uniseriate rays are identical with those of the multiseriate rays. The rays of the stems containing the first two types are classified as heterogeneous and the rays of stems containing the second two types are classified as homogeneous. The question of terminology concerning the multiseriate ray in each type is left to the Committee on Nomenclature. *Heterocellular* is suggested for the ray in which the interior cells are radially elongated and the marginal cells vertically elongated or square; *homocellular*, for the ray in which the cells are all radially elongated.

Ray classification

I. HETEROGENEOUS TYPE 1 (fig. 1)

1. Uniseriate rays usually high, numerous, and composed of very large, vertically elongated cells which are unlike the cells of the multiseriate part of the multiseriate rays.
2. Multiseriate rays usually with parallel sides and with very large, vertically elongated, uniseriate wings (long wings) which are composed of cells identical with those of the uni-

seriate rays. The cells of the multiseriate portion of the ray are oval, radially elongated, or vertically elongated.

II. HETEROGENEOUS TYPE II (fig. 3)

Uniseriate rays usually lower and composed of cells which are unlike those of the multiseriate part of the multiseriate rays.

A: 1. Uniseriate rays composed of rectangular, vertically elongated cells only.

2. Multiseriate rays with sides parallel or fusiform, the cells of the multiseriate portion being round to oval, radially elongated, and with uniseriate tips of large, vertically elongated cells (short wings); or with large, vertically elongated marginals, one cell high.

B: 1. Uniseriate rays of two types; some of the uniseriates are composed of rectangular, vertically elongated cells; some are composed of cells which are nearly identical with those of the multiseriate part of the multiseriate rays.

2. Multiseriate rays with sides parallel or fusiform, the cells of the multiseriate portion being round to oval, radially elongated, and with medium to small, vertically elongated marginals, usually single, occasionally two cells high. If the uniseriate tips are longer, the cells are modified; that is, they are mostly square.

III. HOMOGENEOUS TYPE I (fig. 4)

1. Uniseriate rays rather low, numerous to scarce, and composed of cells which are identical with those of the multiseriate rays.

2. Multiseriate rays mostly fusiform, the cells of the multiseriate portion being round to oval, radially elongated, and with long to short, uniseriate tips composed of cells identical with those of the multiseriate portion of the ray. Occasionally a multiseriate ray possesses very small square marginals, one cell high, which occur sporadically.

IV. HOMOGENEOUS TYPE II (fig. 2)

1. Uniseriate rays usually scarce to absent. When present, low, and composed of cells identical with those of the multiseriate rays.

2. Multiseriate rays fusiform, and composed entirely of small, round, radially elongated cells; uniseriate tips absent or extremely short.

V. UNISERIATE RAYS ONLY: HETEROGENEOUS TYPE III (fig. 5)

VI. UNISERIATE RAYS ONLY: HOMOGENEOUS TYPE III (fig. 6)

Table I shows the percentage of ray types in each vessel type. Scalariform I represents the most primitive type of vessel element; porous-transverse, the most highly specialized type. In stems which

TABLE I

PERCENTAGE OF RAY TYPES IN EACH VESSEL TYPE

Type of vessel element	Number of genera	Ray types					
		Multiseriate and uniseriate				Uniseriate only	
		Heterogeneous		Homogeneous		Heterogeneous type III	Homogeneous type III
		Type I	Type II	Type I	Type II		
Scalariform I	63	**79.36**	15.87	.....	.....	4.77	.....
Scalariform II	32	**53.12**	21.87	12.50	3.13	9.38	.....
Scalariform-porous	67	47.77	**35.81**	5.98	4.48	2.98	2.98
Porous-oblique	220	44.09	**43.18**	5.45	3.56	2.36	1.36
Porous-oblique and transverse	148	9.45	**33.11**	**34.45**	9.49	2.02	11.48
Porous-transverse	220	.....	19.09	**27.28**	**44.10**	1.81	7.72

possess both multiseriate and uniseriate rays, there is a high correlation between ray type and vessel element type; that is, the heterogeneous type I rays predominate in the stems with the scalariform type of vessel element, whereas the heterogeneous type II rays are very conspicuous in the scalariform-porous, porous-oblique, and porous-oblique and transverse groups. On the other hand, the homogeneous type I rays are dominant in the porous-oblique and transverse, and the porous-transverse groups; while the homogeneous type II rays are dominant in the porous-transverse group.

Reading across the table in each vessel element group, the heterogeneous type I rays constitute 79 per cent of the rays in the scalariform I group and 53 per cent in the scalariform II group. In the

scalariform-porous group, nearly 48 per cent are heterogeneous type I rays and nearly 36 per cent heterogeneous type II rays. In the porous-oblique group, the percentages of heterogeneous type I and type II are equal, being 87 per cent of the total number of ray types. In the porous-oblique and transverse group, there is an equal percentage of heterogeneous type II and homogeneous type I rays, or over 67 per cent of the total number of ray types; while in the porous-transverse group more than 71 per cent of the ray types are homogeneous, 44 per cent being homogeneous type II.

Table I shows rather conclusively that the heterogeneous type I rays, the multiseriates with their greatly elongate uniseriate wings, together with the high uniseriates composed of greatly elongate cells, are indeed the primitive type. As specialization continues, the multiseriates become more fusiform in outline, the wings become shorter, and the cells of the uniseriates become smaller, as illustrated by the heterogeneous type II rays. The next step in the evolutionary sequence involves a change from the heterogeneous to the homogeneous condition in which the cells of the uniseriates are identical with those of the multiseriates. In addition, the uniseriates are becoming shorter and less numerous and the multiseriates are mostly fusiform and homogeneous, as illustrated by the homogeneous type I rays. Finally, the most highly specialized condition is represented by the homogeneous type II rays in which the uniseriates are usually scarce or absent, the multiseriates being fusiform and homogeneous. Figure 7 illustrates diagrammatically the salient lines of structural specialization in the wood rays of dicotyledons.

Table II shows the total percentage of ray types in each vessel type. This table also indicates that the heterogeneous type rays are more primitive than the homogeneous ones. In the scalariform I vessel element group, the rays are 100 per cent heterogeneous; conversely, in the porous-transverse vessel element group, the rays are nearly 80 per cent homogeneous.

Tables I and II both indicate that during the process of evolutionary development the primitive type of ray is retained for long periods of time. This is especially true of some of the tropical plants in comparison with those of more temperate regions.

Table III shows a high correlation between ray type and vessel

element length. It indicates that as woody dicotyledonous stems become more highly specialized, the trend is from the heterogeneous type I ray to the homogeneous type II ray, and that heterogeneous rays in general are more primitive than homogeneous ones.

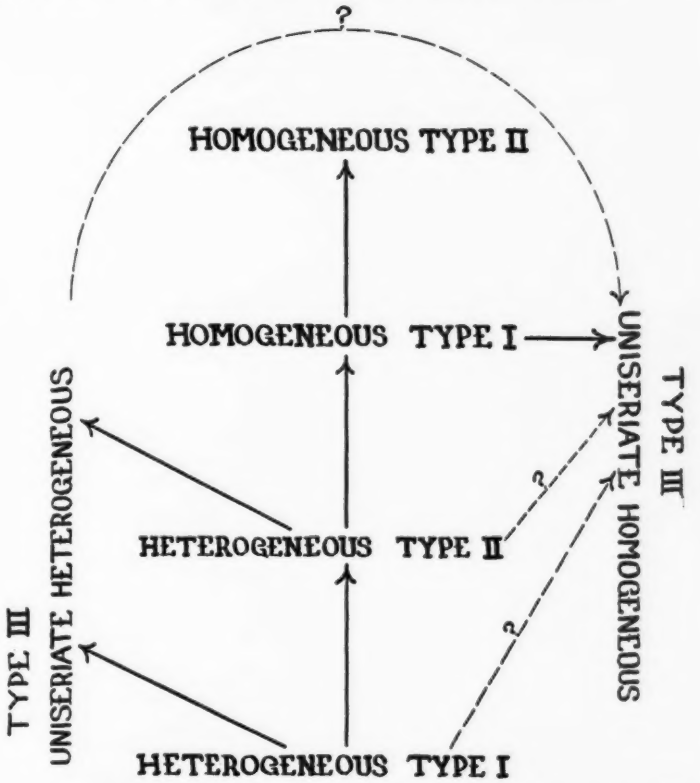

FIG. 7.—Diagrammatic illustration of salient lines of structural specialization in the wood rays of dicotyledons.

Taking into consideration the stems which possess uniseriate rays only, table I shows that there is no correlation between vessel element type and ray type, with the exception that the homogeneous uniseriate ray is more highly specialized than is the heterogeneous uniseriate.

The uniseriate ray occurs as an offshoot in practically every type of woody dicotyledonous stem, indicating that it is a specialized structure due to the elimination of multiseriate rays. BAILEY (1) has

TABLE II

TOTAL PERCENTAGE OF RAY TYPES IN EACH VESSEL TYPE

TYPE OF VESSEL ELEMENT	PERCENTAGE RAYS	
	HETERO-GENEOUS	HOMO-GENEOUS
Scalariform I	100.00	.....
Scalariform II	84.37	15.63
Scalariform-porous	86.56	13.44
Porous-oblique	89.63	10.37
Porous-oblique and transverse	44.58	55.42
Porous-transverse	20.90	79.10

TABLE III

AVERAGE LENGTH OF VESSEL ELEMENT IN MM. FOR EACH RAY TYPE*

TYPE OF RAY	NUMBER OF GENERA	AVERAGE ELEMENT LENGTH (MM.)
Heterogeneous type I	210	0.81
Heterogeneous type II	227	0.58
Homogeneous type I	131	0.52
Homogeneous type II	123	0.35
Uniseriate heterogeneous type III	18	0.64
Uniseriate homogeneous type III	41	0.38

* Vessel element length indicates total element length, tip to tip, from macerated material.

shown this to be true for *Castanea* and *Alnus*, and HOLDEN (10, 11) reached the same conclusions concerning the rays of Salicales and of *Aesculus* in the Sapindales. Furthermore, GROOM (9) has suggested that the small rays of *Quercus* may have originated from the disintegration of primitive wide multiseriate rays. The multiseriate rays of oak, however, are not primitive but specialized structures.

CHATTAWAY (5), in her study of the ontogenetic development of

rays in the Sterculiaceae, also points out that uniseriate rays are derived by the gradual splitting of the multiseriate ray. She states also: "It appears that abundance of sheath-cells and the method of enlarging the rays at the expense of the fusiform initials is a primitive feature, and that the small-rayed species are on the whole more advanced than the large-rayed ones." A lack of material prevented a detailed study of all the genera of the Sterculiaceae; those examined, however, showed no correlation between the presence of sheath cells, size of ray, and ray type. Although some of the large-rayed species in which sheath cells are well developed possess the transitional heterogeneous type II rays, other large-rayed species in which sheath cells are only partially developed possess the same ray type. This is also true of some of the small-rayed species in the same category. The same results were obtained for the woods of group B, rays normally without sheath cells. The presence of sheath cells seems to indicate a distinct line of specialization restricted to the subfamily Sterculieae. This also seems to be true for tile cells in the rays of certain of the Malvales.

Summary

1. Rays of woody dicotyledons may be classified into six salient types: heterogeneous type I; heterogeneous type II; homogeneous type I; homogeneous type II; uniseriate, heterogeneous type III; uniseriate, homogeneous type III.

2. There is a high correlation between vessel type and ray type, the evolutionary sequence being from the heterogeneous type I, the most primitive, through the transitional heterogeneous type II and homogeneous type I, to the highly specialized homogeneous type II rays.

3. The uniseriate, homogeneous type III rays are more highly specialized than the uniseriate, heterogeneous type III rays.

4. The uniseriate ray is a highly specialized structure owing to the elimination of multiseriate rays.

5. Specialization of rays is dependent upon the degree of heterogeneity rather than upon the size of the rays.

6. The so-called compound ray is merely an unusually wide multiseriate ray. Such rays are of common occurrence in both primitive

and specialized types of dicotyledons, and may be of either the heterogeneous or the homogeneous type.

7. The so-called aggregate ray is a specialization which occurs sporadically. It is an offshoot from the main line of structural specialization in rays.

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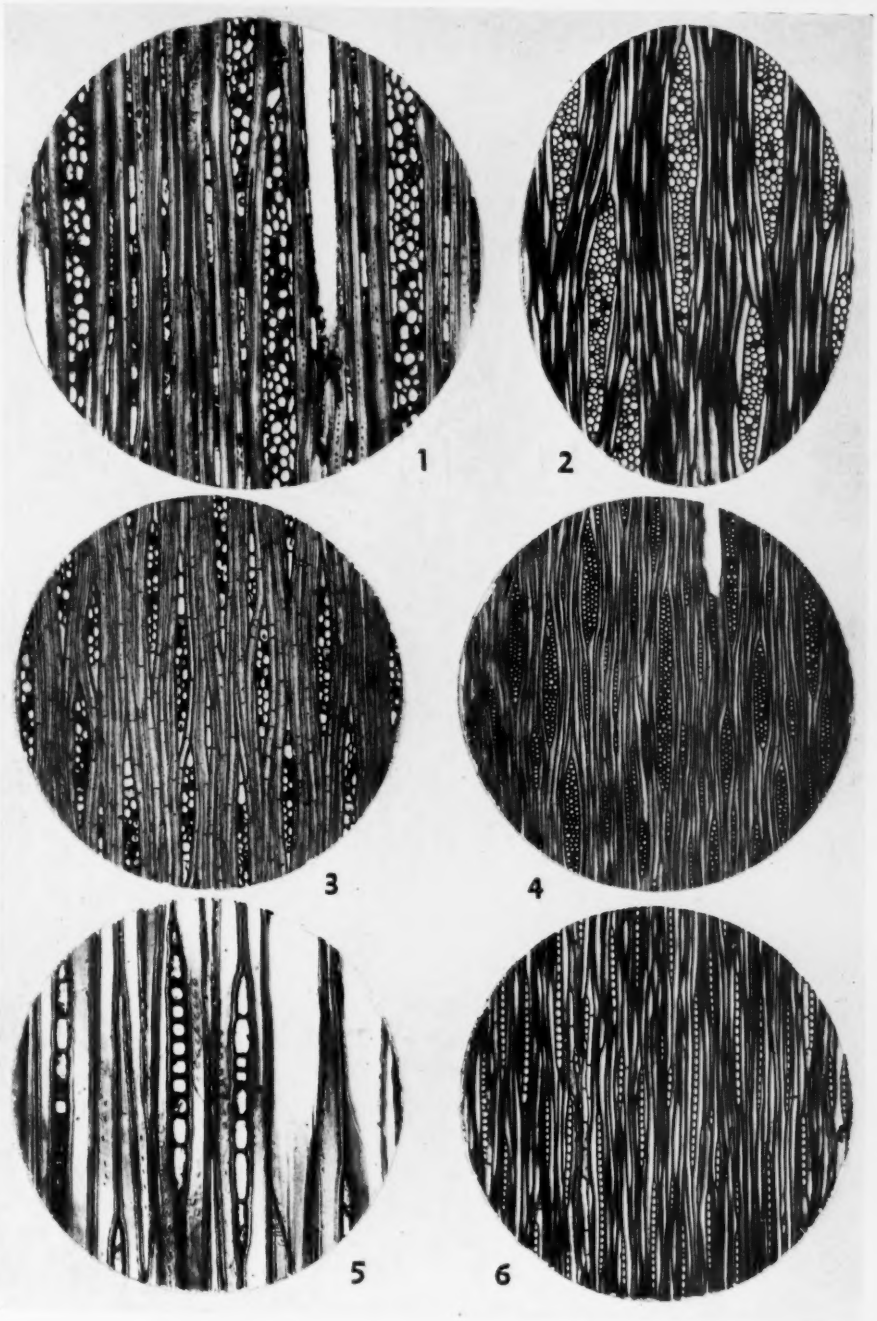


KRIBS on WOOD RAYS

EXPLANATION OF PLATE VIII

FIG. 1. *Taonabo peduncularis* (DC.) Pittier. Tangential longitudinal section of xylem, illustrating type I heterogeneous rays. ×62.

FIG. 2. *Crataeva tapia* L. Tangential longitudinal section of xylem, illustrating type II homogeneous rays. ×62.

FIG. 3. *Dracontomelum dao* Merr. & Rolfe. Tangential longitudinal section of xylem, illustrating type II heterogeneous rays. ×62.

FIG. 4. *Acer mandshuricum* Maxim. Tangential longitudinal section of xylem, illustrating type I homogeneous rays. ×62.

FIG. 5. *Schima confertiflora* Merr. Tangential longitudinal section of xylem, illustrating type III heterogeneous rays. ×158.

FIG. 6. *Castanea pumila* (L.) Miller. Tangential longitudinal section of xylem, illustrating type III homogeneous rays. ×62.

SOMATIC CHROMOSOME NUMBERS IN THE GENUS SEDUM

J. T. Baldwin, Jr.

(WITH FOURTEEN FIGURES)

Introduction

This note is written as a preliminary report on a genetic and cytological study of the genus *Sedum*. The results ought to be of value in clearing up some of the problems of classification and of evolution within the group. Such application of the results from cytogenetic investigations is in accord with the more recent developments in taxonomic work.

No reports of genetic studies on any of the Crassulaceae and no references to extensive cytological investigations of this family have been encountered. Rocén (**5**) states that determinations of embryological relationships in the Crassulaceae are relatively few in comparison with those made for several other families of the order Rosales. The standard lists of chromosome numbers in plants contain few chromosome counts for this family; Gaiser (**2**) records only the following:

	n	2n	
Bryophyllum calycinum Salisb.	40	(38?)	Taylor (1926)
Penthorum sedoides L.	8		Rocén (1928)

Sedum appears to be a genus suitable for cytogenetic investigation. Most species of the genus grow well from seed and may be transplanted without their growth being seriously checked. Most of the species are easy to propagate vegetatively; by this means plants of particular interest may be preserved. Annuals, biennials, and perennials exist; monoecious and dioecious species are known. The majority of the cultivated forms are hardy in our climate. Natural species hybrids, although rare, occur. A wide range of form, size, and color, both flower and foliage, is available for genetic analysis. The chromosome number, although large, has been found to vary

from species to species, and in one case within a species. The chromosomes of a complement, although the chromosomes are small, have been observed in root tip metaphases to be of a range of sizes. In many cases chromosome constrictions have easily been detected. These, with still other characteristics, would seem to recommend *Sedum* as favorable material for study.

According to HUBER (3), *Sedum* is the most widely distributed genus of the Crassulaceae; to this family BAILEY (1) assigns twenty genera. PRAEGER (4) estimates the genus to include about 500 known species, which are found in varying abundance throughout the Northern Hemisphere and below the equator in the Andes of Peru and Bolivia. The main habitat is central Asia, especially the mountains of West China. Ten generic subdivisions, established for the most part on general growth form, are recognized by PRAEGER (4). HUBER (3) observes that the geographic distribution of the genus coincides in the main with its morphological and systematic arrangement. To ascertain the relationships existing between the sections of the genus and their chromosome numbers, therefore, will be of interest from the viewpoint of the evolution of the various groups. In other plant genera a knowledge of chromosome numbers and of chromosome morphology has been helpful in determining species and in discovering interspecific relations. These two criteria of taxonomic position, applied on the basis of the systematic work already done on the groups, should be of much value in so polymorphic a genus as *Sedum*.

Taxonomically *Sedum* is not a particularly difficult genus. Some of its species are variable but the greater part of them are stable and distinct. In so far as its cultivated forms are concerned, however, *Sedum* is in a much confused state. In large measure this confusion is caused by the ease with which the plants propagate themselves asexually, thereby frequently spreading to areas supposedly prohibited to them, and by the dispersal, through growers, of all plants from a given plot as belonging to the form to which the plot had been assigned. This and the occurrence of names which have been given to no described species contribute much to the incorrect identification that is prevalent.

Material and methods

Root tips were obtained from the adventitious roots of plants growing in the garden or of plants placed over water in the laboratory. They were fixed in Nawaschin's solution, cleared in xylene and imbedded in paraffin, sectioned at a thickness of 7 μ, and stained according to Newton's gentian violet method. Fixation was usually good.

All drawings were made at table level with the aid of a camera lucida and at a magnification of $\times$4900. A Zeiss microscope, equipped with a $\times$30 compensating ocular and a $\times$90 fluorite objective, n.a. 1.25, with yellow-green filters, was used.

Results

The somatic chromosome numbers of twelve *Sedum* species, distributed among four sections of the genus, have been determined. Specific identifications assigned by growers have been checked with the descriptions found in PRAEGER (4) and HUBER (3). A form of *S. hispanicum* L. was obtained from the Valley View Greenhouses, Charlottesville, Virginia, as *S. glaucum*. The latter is a synonym for the former (Index Kewensis). Plants collected from the wild or from private gardens have likewise been identified except in two instances. One of the unidentified forms resembles *S. purpureum* Link; the other resembles *S. telephium* L. PRAEGER (4) places *S. purpureum* as a subspecies of *S. telephium*. The species investigated, the sections to which they belong, their somatic or 2n chromosome numbers, and the sources from which the plants were obtained are given in table I.

The chromosomes in all the root tip metaphases examined were usually well spaced and found in one plane. As a result the counting was not difficult, except in some of the higher numbered forms in which it was not always easy to distinguish constrictions from actual separations.

Discussion

The chromosome numbers of the species so far investigated form an irregular series apparently founded upon no common basic number. A regular series, however, is shown by the forms in one of the generic subdivisions investigated.

Section Telephium is composed of a group of about 25 hardy

perennial species which range across Eurasia from England to Japan. They are most abundant in the East. One species, however, *S. telephioides*, occurs in and is restricted to North America. The four forms of this group now investigated exhibit a chromosome

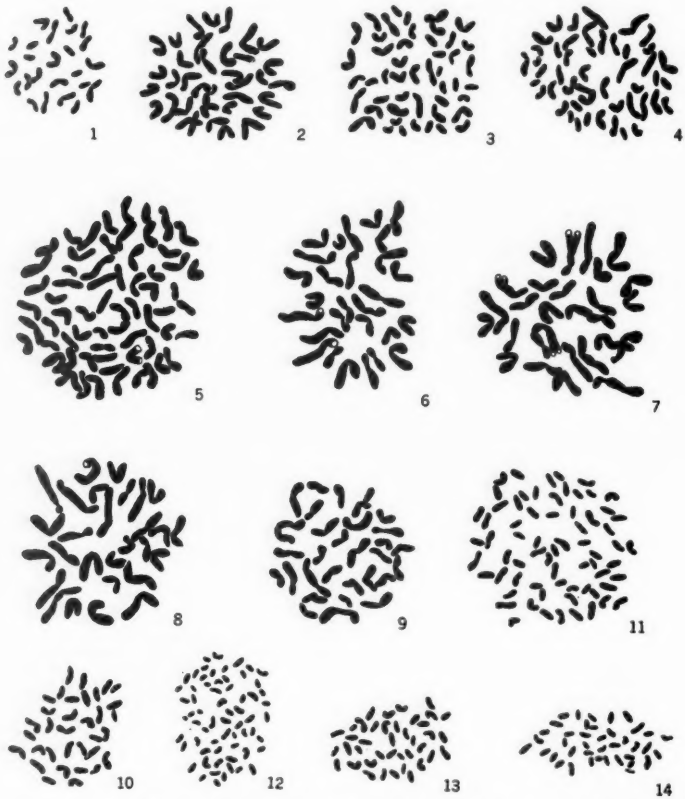
TABLE I

Section	Species	2n chromosome number	Locality
Telephium S. F. Gray	S. telephioides Michx.	24	Wild, Shepherdstown, W. Va.
	S. telephioides Michx.	24	Ridgeville Nurseries Inc., Mount Airy, Md.
	Sedum sp. (resembles S. purpureum Link)	36	Cultivated, The Plains, Va.
	Sedum sp. (resembles S. telephium L.)	48	Cultivated, Charlottesville, Va.
	S. spectabile Boreau	48	E. B. Drake Alpine Gardens, Lakebay, Wash.
Aizoon Koch	S. kamtschaticum Fisch and Meyer	64	Valley View Greenhouses, Charlottesville, Va.
Seda Genuina Koch	S. divergens (?) S. Watson	28	" "
	S. spurium M. B.	28	" "
	S. stoloniferum S. T. Gmelin	28	" "
	S. ternatum Michx.	32	Wild, Boyce, Va.
	S. reflexum L.	34	Ridgeville Nurseries Inc., Mount Airy, Md.
	S. reflexum L.	68	Nursery near Washington, D.C.
	S. album L.	64	" "
	S. album L.	64	Valley View Greenhouses, Charlottesville, Va.
Epeteium Boissier	S. hispanicum L.	40	E. B. Drake Alpine Gardens, Lakebay, Wash.
	S. hispanicum L.	40	Valley View Greenhouses, Charlottesville, Va.

series with a basic number of 12. *S. telephioides* has been found to have as a 2n number 24 chromosomes (fig. 1); *Sedum* sp. from The Plains, Virginia (possibly cultivated from the wild), has 36 (fig. 2); *Sedum* sp. from Charlottesville, Virginia (possibly from the wild), has 48 (fig. 3); and *S. spectabile* has 48 (fig. 4). Thus a series of 24–36–48 is present.

Only one chromosome number, that of *S. kamtschaticum* with 64

chromosomes (fig. 5), has been determined for section Aizoon. This section includes only a few species, which are exclusively limited to eastern Asia, and which give a fairly uniform impression.



FIGS. 1–14.—Metaphase plates from root tips of *Sedum:* fig. 1, *S. telephioides* with 24 chromosomes; fig. 2, *Sedum* sp., 36; fig. 3, *Sedum* sp., 48; fig. 4, *S. spectabile*, 48; fig. 5, *S. kamtschaticum*, 64; fig. 6, *S. divergens?*, 28; fig. 7, *S. spurium*, 28; fig. 8, *S. stoloniferum*, 28; fig. 9, *S. ternatum*, 32; fig. 10, *S. reflexum*, 34; fig. 11, *S. reflexum*, 68; fig. 12, *S. album*, 64; fig. 13, *S. hispanicum*, 40; fig. 14, *S. hispanicum*, 40. ×2450.

Section Seda Genuina lacks a natural relationship, for in it are associated a number of forms that fit into none of the other groups. It has a wider distribution range and includes more species than any

other section of the genus. Six species of this section have been investigated. They are *S. divergens?* with 28 somatic chromosomes (fig. 6); *S. spurium*, 28 (fig. 7); *S. stoloniferum*, 28 (fig. 8); *S. ternatum*, 32 (fig. 9); *S. reflexum*, 34 (fig. 10) and 68 (fig. 11); and *S. album*, 64 (fig. 12). The 68-chromosome-numbered form of *S. reflexum* is more branched and less compact than the 34-chromosome-numbered form of the species. No progressive series of chromosome numbers can at present be arranged for the group as a whole.

Section Epeteium, made up of annual, rarely biennial, forms, is widely distributed. Only two forms of this section have been examined. They belong to *S. hispanicum*, and each has 40 somatic chromosomes (figs. 13, 14).

The chromosomes of none of the other six sections of the genus have yet been investigated. With the exception of section Rhodioli, which is scattered over most of the mountains of the north temperate zone, these other sections are small and restricted in their distribution.

Because of insufficient data, no comparison with work on other genera and no discussion of chromosome morphology will at present be attempted. Investigations now in progress will make such a treatment possible and will be reported in a later paper.

Summary

1. Little cytogenetic research has been done on the Crassulaceae. The genus *Sedum* is good material for study.

2. The somatic chromosome numbers of twelve *Sedum* species, including fourteen forms distributed among four generic sections, are reported.

3. A chromosome series of 24–36–48, with a basic number of 12, is exhibited by the forms reported in section Telephium S. F. Gray.

The writer wishes to express his appreciation to Dr. Orland E. White for his valuable criticisms during the course of this study and for making available the material with which the investigation is concerned.

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CHROMOSOME NUMBERS OF SOME OF THE CACTACEAE

PALMER STOCKWELL

(WITH SEVENTEEN FIGURES)

Continuing his interest in the cacti (**8**), the writer has begun a cytological investigation of them, believing that studies of the chromosome numbers of this group might aid in the solution of its taxonomic problems. This paper is a report of preliminary findings in this direction.

Root tips of available species were fixed in a chromo-acetic-formalin mixture used in this laboratory. This formula is designated as CL_9 and is as follows:

SOLUTION A, 2 PARTS	SOLUTION B, 1 PART
100 cc. distilled water	0.5 gm. saponin
10 cc. glacial acetic acid	100 cc. 16% formalin solution
1 gm. chromic acid	
1 gm. potassium dichromate	
1 gm. urea	

(Mix solutions A and B immediately before use.)

The slides were stained with gentian violet, safranin, and orange G as described in a previous paper (**7**). With this procedure no particular difficulty was encountered in counting the chomosomes.

Eight species or forms of *Opuntia*, one of the more primitive groups of the cacti, were examined. These plants of the flat-jointed, prickly pear type had been transplanted from their natural habitats and kept in cultivation for different periods of time varying from a few months to several years. Of the tribe Cereeae, a highly evolved group, nine species were investigated, most of them being greenhouse seedlings. They included specimens of the giant cactus of the southwestern United States (*Carnegia*); a giant cactus of northern Mexico (*Lophocereus*); one of the Christmas cacti, an epiphytic form from Brazil (*Schlumbergeria*); one of the porcupine cacti of the California

desert (*Echinocereus*); and several pincushion cacti from California, Arizona, and Texas (*Neomammillaria*). The specimens of *O. polyacantha* were collected by Dr. GÖTE TURESSON of Lund, Sweden, during a visit to this country last year. A number of the other plants were provided by Professor JAMES I. MCMURPHY of Stanford University.

The known somatic chromosome numbers in the Cactaceae are recorded in table I. Those counted in this study are numbered 1 to 17 in the table and are also illustrated in figures 1–17. The species are arranged after the system of BRITTON and ROSE (1). The chromosomes of the species investigated are rather small and polyploidy seems to be a common phenomenon, with 11 as the most frequent basic chromosome number. The species within the tribe Cereeae are morphologically more closely related to one another than to any species of the tribe Opuntieae, and *vice versa*. This relationship is also indicated in the chromosome plates. In general the Cereeae have larger chromosomes and a smaller number of them. Furthermore, the basic chromosome number appears to be more variable in the tribe Cereeae than in Opuntieae.

In Cereeae the diploid number 24 was reported by SUGIURA (9). In the nine species of this tribe examined in the present investigation, 44, 22, or 18 chromosomes were found (nos. 9–17, table I).

In the tribe Opuntieae, JOHANSEN (5) has reported a diploid number of 22. In the eight species of this tribe here examined the numbers 66, 44, or 22 have been observed (nos. 1–8, table I).

So far as we know, therefore, the basic numbers of the tribe Cereeae are 9, 11, and 12 while the basic number 11 is the only one reported for the tribe Opuntieae. Large satellites were found in representatives of both tribes when the chromosome number was low, but they have not been observed in those having high chromosome numbers. The satellites of *Lophocereus schottii* are so large that one may suspect that they are constricted ends of the chromosomes themselves.

Generalizations based on the scanty data at hand are hazardous. Some interesting conjectures, however, may be made from a projection of the results obtained to date. The opuntias are the most northern of the cacti and they have the higher chromosome num-

bers. In the species *Opuntia polyacantha*, chromosomes have been counted in specimens from Colorado, Saskatchewan, southern Alberta, and Peace River Crossing in northern Alberta near the station

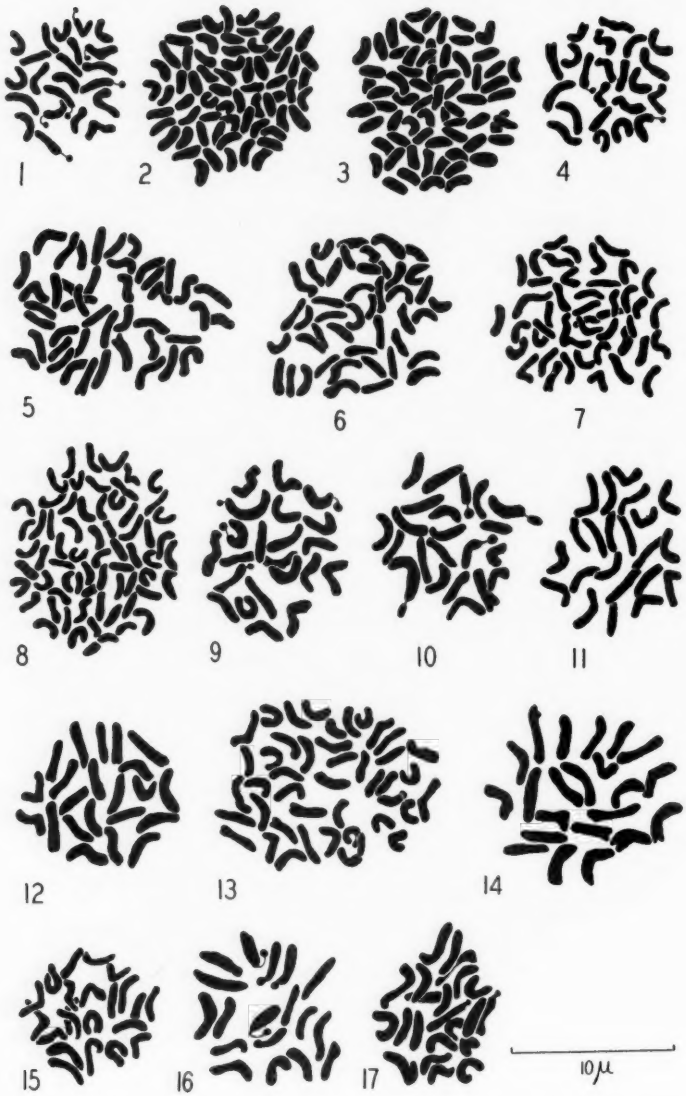
TABLE I

KNOWN SOMATIC CHROMOSOME NUMBERS IN CACTACEAE

FIGURE NO.	NAME	APPROXIMATE DISTRIBUTION	2n
	Tribe Opuntieae		
1	Opuntia santa-rita (G. & H.) Rose	Southern Arizona and northern Mexico	22
2	O. phaeacantha Engelm.	Texas to California and northern Mexico	±66
3	O. discata Griffiths	Southern Arizona	±66
4	O. chlorotica Engelm.	New Mexico to California	22
5	O. polyacantha Haw.—Colorado Springs	New Mexico to California and north to Canada	44
6	O. polyacantha Haw.—Saskatoon, Saskatchewan	New Mexico to California and north to Canada	44
7	O. polyacantha Haw.—Southern Alberta	New Mexico to California and north to Canada	44
8	O. polyacantha Haw.—Peace River, northern Alberta	New Mexico to California and north to Canada	±66
*	O. brasiliensis (Willd.) Haw.	Brazil	22
	Tribe Cereeae		
9	Carnegia gigantea (Engelm.) B. & R.	Arizona and northern Mexico	22
10	Lophocereus schottii (Engelm.) B. & R.	Northern Mexico	22
11	Echinopsis multiplex (Pfeif.) Zucc.	Brazil	22
†	Zygocactus truncata Schum.	Brazil	24
12	Schlumbergeria russelliana (Gard.) B. & R.	Brazil	22
13	Echinocereus engelmannii (Parry) Rump.	New Mexico to California and northern Mexico	44
14	Ferocactus rostii B. & R.	Southeastern California	22
15	Neomammillaria macdougalii (Rose) B. & R.	Arizona	22
16	N. applanata (Engelm.) B. & R.	Texas	18
17	N. fragilis (Salm-Dyck) B. & R.	Mexico	22
†	N. glochidiata (Mart.) B. & R.	Southern Mexico	24

* Counted by JOHANSEN (5).
† Counted by SUGIURA (9).

for the northernmost collection of cactus known. Of these, the northernmost form, much smaller than its more southern relatives and with smoother pads and fewer spines, had the somatic number 66



FIGS. 1–17.—Somatic chromosome plates from root tips of various cactus species. The numbers correspond to those of table I.

while the remainder had 44. This fact bears out the observation of HAGERUP (**2**): "It is worth noting that among these four pairs of species (including *Empetrum nigrum* and *E. hermaphroditum*) those with the higher chromosome numbers are always the ones growing farther north." But it is probable that in the *O. polyacantha* from Peace River the larger chromosome number merely enables the plant to grow locally where it could not otherwise survive.

Polyploidy with the ensuing greater possibilities for variation through changes in the chromosome set-up may furthermore enable a species to occupy a more extensive territory. Of the Cereeae examined, seven had 22 chromosomes, one had 18, and one had 44. The geographical area of these plants is usually not so great as that of the opuntias, but it is interesting to note that *Echinocereus engelmannii*, with its various forms, has the greatest distribution and the largest chromosome number. Among the opuntias, *O. polyacantha*, the northern species with 44 and 66 chromosomes, is the most widely distributed of those here examined. *O. phaeacantha* also with 66 chromosomes is southern, but its geographical range is great. *O. discata* with the same number is restricted in area but it seems to be morphologically only a localized form of the widely distributed species *O. engelmannii*, of which no material was available. Of the two opuntias having 22 chromosomes, the range of *O. santa-rita* is limited while *O. chlorotica* is widespread but sporadic in its occurrence and it seems to be rather selective in its environmental requirements. These facts would indicate that there may be a correlation between distribution and polyploidy. This is in harmony with NAWASCHIN'S (**6**) statement that, "through change in the rate of development, a polyploid individual may acquire the ability of withstanding different climatic conditions. As a consequence it may penetrate into a new territory. . . . ." This view is concurred in by HAGERUP (**4**), who states, "polyploid forms may be ecologically changed so as to grow in other climates and formations where the diploid form will not thrive." His work with desert plants (**3**) bears out this statement as in some cases polyploidy increased under increasingly adverse conditions.

Inasmuch as cacti range from considerably below the equator in South America to 56° north latitude, are epiphytic in the tropical

rain forests, and are among the few representatives of the plant kingdom in certain parts of the desert, it is expected that a more exhaustive research from this viewpoint may prove a valuable approach to the problems of plant distribution and the species question.

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MATERNAL TRANSMISSION OF MUTATED PLASTIDS IN THE JAPANESE MORNING GLORY

KIICHI MIYAKE AND YOSHITAKA IMAI

(WITH TWO FIGURES)

Chlorophyll variegation, which is commonly found in the Japanese morning glory (*Pharbitis nil*), is transmitted as a simple recessive to the self-green condition. The whitish patches appear somewhat irregularly on the respective leaves of variegated individuals, so that the variegation is regarded as a pattern character. The allelomorphs, normal (self-green) and variegated, are quite constant and no cases of mutations either from normal to variegated or its reverse have come under our notice.

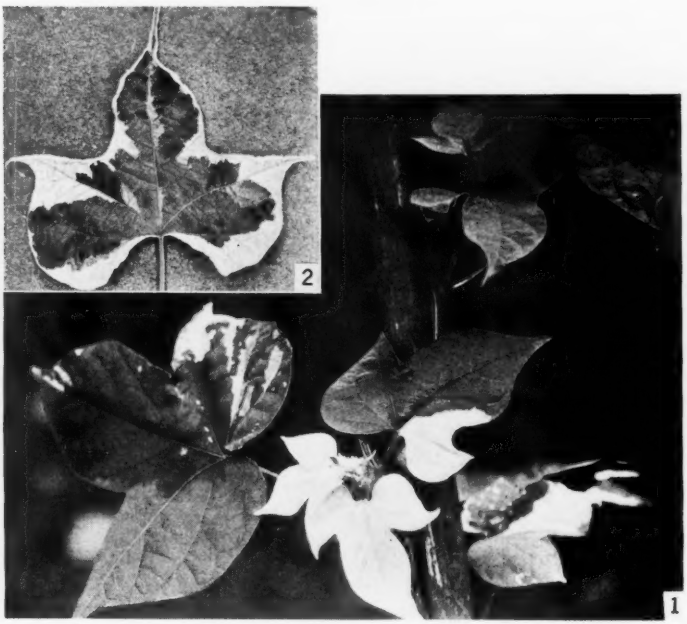
In 1928 three unexpected variants with variegated leaves incidentally occurred in the hybrid progeny of different crosses, their sister plants being invariably self-green. The variegation of these plants differed in color, namely:

1. White; variegation in white.
2. Creamish; at first cream in color and creamish white in the extended leaves.
3. Yellowish; variegated with yellowish color, a little more intense in the young leaves.

The white-variegated variant puts out at times green and white branches (fig. 1) and also chimerical bud variations with periclinal white-over-green tissues. The results obtained from selfing the flowers of these branches are collected in table I.

The albinotic seedlings had short hypocotyls with small cotyledons and perished about two weeks after germination. The green individuals thus segregated bred true to type for generations, while the variegated ones gave rise to three forms in various proportions according to the amount of variegation of the mother plants or of the branches from which the seeds were collected. The slightly variegated branches or individuals gave mostly green seedlings, whereas the highly variegated ones produced mostly albinotic seed-

lings. The variegated segregates were at all events few in proportion compared with the green and white segregates. The chimerical branches shown in table I were of green "cores" with white "skins" (fig. 2); as expected, they produced only albinotic seedlings.



FIGS. 1, 2.—Fig. 1, white-variegated plant, with green (upper right) and white (lower left) branchlets. Fig. 2, chimerical leaf with green core covered with white skin, taken from progeny of the original variegated. The crumpling of the leaf is due to the gene crumpled-1.

At the same time the original variegated plant was crossed with a self-green strain in reciprocal ways, the results being given in table II.

Table II shows that the case is evidently non-Mendelian owing to plastid inheritance. Five green plants obtained by cross green ♀ × variegated ♂ were selfed and they bred true to type in the subsequent generation, as expected. In experimenting with the other variegated mutants, the same procedure was followed, when it was

found that both creamish and yellowish plastids were inherited maternally out of Mendelian rule. Up to the present a considerable number of cases of plastid inheritance have been studied, and recently DE HAAN (1) made a collection of available data to that date, to which he added his own criticisms.

The origin of the variegation in these variants of the Japanese morning glory is due to sporadic occurrences of plastid mutations.

TABLE I

OFFSPRING OF WHITE-VARIEGATED VARIANT, SHOWN SEPARATELY ACCORDING TO TYPES OF BRANCHES

CHARACTER OF BRANCH	GREEN	VARIEGATED	ALBINOTIC	TOTAL
Green	69	.....	.....	69
Variegated	168	10	38	216
White	.....	.....	28	28
Chimerical	.....	.....	15	15

TABLE II

OUT-BREEDING DATA OF WHITE-VARIEGATED VARIANT

CROSS	GREEN	VARIEGATED	ALBINOTIC	TOTAL
Green×white	66	.....	.....	66
White×green	.....	.....	24	24
Green×variegated	48	.....	.....	48
Variegated×green	25	3	19	47

Let us suppose that one of the green plastids, contained in an embryonic cell of the original white-variegated plant (variegation had appeared already at the seedling stage), had mutated to a white one and had a chance to propagate in a cell or cells of the growing point of the seedling. This is one of the most likely explanations to account for the origin of the variant. For the other two cases of variegation, similar mutations would take place, but from green to creamish or yellowish and possibly at different times of somatogenesis (variegation appeared later when the plant began to take on a luxuriant growth). In these cases the plastid mutations were sporadic in their occurrence (not mutable) and the mutated plastids never reverted to their prototype so far as we could observe. Prior to this, IMAI

(2) observed plastid mutations in this plant and wrote: "I have met with two or three cases, in which the variegated leaves or branches were produced on self-green plants, in my own culture. The appearance of variegation on the self-coloured leaves was due not to a factor mutation, but to a plastid mutation, their transmission, therefore, being non-Mendelian." In another publication (3) he gave a short note on the occurrence of the three forms of plastid mutations which we are investigating at present. In Asagao-Sô, a classical monograph of the Japanese morning glory published in 1817, we find two colored pictures of different specimens, each showing a mosaic of green and variegated colorations of leaves (2, figs. 6 and 7). Although, as pointed out by IMAI, the colors are somewhat exaggerated, the depiction of such mosaics is no doubt explained by the fidelity of the earlier authors to facts in their drawings and descriptions. Two possible explanations may be suggested to account for the origin of the mosaic specimens; namely, gene mutations occurring vegetatively from green to variegated, or plastid mutations from green to white. As the chances for the former seem more remote than the latter in this plant, we are inclined to accept the latter view also in these classical cases.

The expenses of the present research were partly defrayed by a grant from the Imperial Academy, to which we wish to express our cordial thanks.

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3. IMAI, Y., The genetics of *Pharbitis Nil*. Jap. Jour. Genetics 6:129–131. 1930. (In Japanese.)

CURRENT LITERATURE

Excretion in the higher plants

Following a suggestion of the late Professor KOSTYTSCHEW, FREY-WYSSLING[1] has written a monograph on the excretory and secretory processes of the higher plants. It is volume 32 of the Monographien aus dem Gesamtgebiet der Physiologie der Pflanzen und der Tiere. The products of assimilation and dissimilation are so numerous and varied that the work covers a wide range of material and many diverse processes. Even then the work will be found not to have covered all of the wastes and secretions that are formed in metabolism.

The author has not confined his attention strictly to the excretion and secretion phenomena, but surveys structural, physiological, and even phylogenetic problems that throw light on these processes of waste removal and deposition of metabolites. The work is the more useful and stimulating, however, for this well rounded and broad treatment.

The introduction sets forth the general relationship of such processes as absorption (resorption), assimilation, and dissimilation to excretion, secretion, and "recretion." True excretion follows dissimilation, while secretion is related to assimilation. Recretion is a term used to express the removal of absorbed (resorbed) mineral elements which are obtained from the soil, and which function in the active protoplasm for a time, but without being assimilated. These minerals are finally deposited by dehydration, precipitation, diffuse deposition in cell walls, or are removed by guttation.

There are four main sections to the work as follows: (1) cell wall; (2) recretion; (3) excretion; and (4) secretion. A brief summary, literature list, and subject index conclude the monograph.

In connection with the cell wall discussion, a great deal of attention is given to submicroscopic structures such as micellae, intermicellar spaces, and intermicellar deposits. The physiological significance of the walls also is considered at some length, such subjects as swelling of walls, their elasticity, plasticity, growth, and permeability and semipermeability relations being given detailed treatment.

The section on recretion deals mainly with the calcium salts (oxalate and carbonate) and silicic acid. Here again such physiological processes as absorption of minerals, ion exchange, selective absorption, and the inability of plants to exclude materials in the environment of the roots are brought in to widen the horizon of the discussion.

[1] FREY-WYSSLING, A., Die Stoffausscheidung der höheren Pflanzen. 8vo. pp. xii+378. Springer, Berlin. 1935.

The excretions are all terpenes or their derivatives, such as isoprene, ethereal oils, balsams, camphor, resins and resin acids, crocetin, vitamin A, vetulin, sapogenin, carotenoids, and caoutchouc.

The last chapter, on secretions, considers the external secretions such as are produced by root hairs, haptotropic organs, nectaries, digestive glands, etc.; and some of the internal secretions, particularly the secretion of enzymes and hormones of various kinds.

Plants are in sharp contrast to animals in that most of their waste materials are deposited within their own bodies. Without important excretory organs, plants have met this problem of existence as effectively as animals. This monograph is a timely and valuable summary of a somewhat neglected field of plant physiology.—C. A. SHULL.

Statistical methods for research workers

The fifth edition of FISHER'S[2] monograph appeared late in 1934, thus maintaining the policy of frequent editions of this work. This edition shows only a few changes from the preceding one. Section 5 is a historical note on the main contributors to statistical theory; two new sections, 21.01 on YATES' correction for continuity and 21.02 on the exact method for 2×2 tables, have been inserted in the chapter on goodness of fit; and section 49.1 dealing with the analysis of covariance has been revised and slightly enlarged.

Section 29.1 at the close of the chapter on significance of means gives methods for the omission of an independent variate when it is found that such variate may with advantage be omitted. To be able to do this in a simple fashion should encourage workers to include more independent variates in their studies than has been customary in the past. If they are found to be insignificant, it is relatively easy to omit the useless variates after the calculations have been made, without complicated recalculations. The work is an extremely useful guide to the problems of biological statistical analysis.—C. A. SHULL.

Silviculture

The continued interest in the teaching and practice of silviculture is shown by the appearance within less than a year of two texts on the subject. The first[3] is really a third edition of a standard text rewritten and brought up to date. The earlier edition was noticed in this journal,[4] and its success is indicated by the demand for a revised edition after an interval of 15 years. While intended as a guide to technical foresters, it shows clearly the application of the principles

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[2] FISHER, R. A., Statistical methods for research workers. 5th ed. 8vo. pp. xiii+319. Oliver and Boyd, Edinburgh and London. 1934.

[3] HAWLEY, R. C., The practice of silviculture with particular reference to its application in the United States of America. 3d ed. 8vo. pp. xv+340. figs. 67. John Wiley & Sons, New York. 1935.

[4] BOT. GAZ. **87**:668–669. 1920.

of ecology to the development of economic forest. A desirable feature of this text is the list of references at the close of each chapter that may serve to guide the reading of the forester.

The second volume[5] is somewhat broader in its scope and embraces both the theory and the practice of forest production. The five parts into which the book is divided indicate the breadth of its scope. They are: plant physiology, forest ecology, systematized silvicultural experience, the forest itself as a source of silvicultural knowledge, and silvicultural literature. The earlier parts analyze the factors which constitute forest environment and the latter apply the results of this analysis to the problems of forest production. Both books have come from ripened experience in two of our leading schools of forestry, and will prove valuable aids to teachers and students.—G. D. FULLER.

The Botanical Review

A botanical magazine[6] edited and published with a new purpose and on a new plan has made its appearance. It is to be issued monthly and the first number appeared in January, 1935. Each issue is to contain two or three articles summarizing and evaluating all recent important contributions in that particular portion of the botanical field. Eventually all phases of botany will be considered. Each article will be written by an expert authority at the invitation of the editors, unsolicited manuscripts not being accepted. The yearly volumes will consist of about 400 octavo pages.

The journal is owned and edited by H. A. GLEASON and E. H. FULLING of the New York Botanical Garden, who have associated with them a board of advisory editors whose scientific attainments guarantee a high standard for the journal. It is printed by the Science Press Printing Company.

The January and February issues contain: Possibilities in plant virus classification, by L. O. KUNKEL; The structure of protoplasm, by WILLIAM SEIFRIZ; Glacial and postglacial vegetation, by PAUL B. SEARS; The structure of the walls of higher plants, by DONALD B. ANDERSON.

It would seem that such a journal should have a wide circulation and prove particularly useful to botanists who have access to only limited library facilities. It should certainly prove time-saving to all.—G. D. FULLER.

Day lilies

Some of these attractive plants, species of *Hemerocallis*, have become so completely naturalized in parts of the United States that they are almost regarded

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[5] BAKER, F. S., Theory and practice of silviculture. 8vo. pp. xiv+502. figs. 87. McGraw-Hill, New York. 1934. $5.

[6] The Botanical Review. H. A. GLEASON and E. H. FULLING, editors and publishers. New York Botanical Garden, Bronx Park, New York and Lime & Green Streets, Lancaster, Pa. $3.00 per year; issued monthly.

as natives. STOUT,[7] however, is convinced that all truly wild day lilies are confined to Asia, where their range is from Manchuria to the Caucasus. Probably other wild species, hitherto unknown, may be brought from the Orient into cultivation and added by plant breeders to the existing horticultural varieties.

The present volume describes both the botanical species and the cultivated varieties. It is illustrated by numerous plates, several of them in color, and many from the larger collection grown in the New York Botanical Garden. An unusual feature of the book is the brief biographical sketches, together with the addresses, of the principal plant breeders and florists who have hybridized and developed these plants.—G. D. FULLER.

Botany in Poland

A recent sketch[8] of the history of the development of botany in Poland will tend to give that country the recognition it deserves. With the usual beginning with the herbals of the 16th century, six different periods are recognized. Sketches of the contributions of individual botanists are enlivened by portraits, beginning with that of SZYMON SYRENSKI (SYRENIUS), 1540–1611, and ending with those of living botanists. Emphasis is placed on the rapid advancement made since 1918.—G. D. FULLER.

Moss flora of North America

The final part of GROUT'S work[9] on the pleurocarpous mosses (volume III) appeared recently. Parts 1[10] and 2[11] have already been reviewed in this journal. Besides completing the family Leskeaceae, part 4 also contains the families Hypopterygiaceae, Hookeriaceae, Neckeraceae, Meteoriaceae, Pterobryaceae, Leucodontaceae, Cryphaeaceae, Fabroniaceae, and Fontinalaceae.

The genera *Pseudoleskea* and *Leskea* of the family Leskeaceae were studied by A. J. SHARP, and the family Fontinalaceae by WINONA H. WELCH. In the latter monograph WELCH has constructed keys based chiefly upon vegetative characteristics, a valuable feature since the water mosses are often collected when not in fruit.

Adequate keys to the genera of each family and to the species of each genus are provided. Synonyms, descriptions, citations of previous illustrations, exsiccati, and varieties are given for each species. A complete index to the

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[7] STOUT, A. B., Day lilies. The wild species and garden clones, both old and new, of the genus *Hemerocallis*. pp. vi+119. pls. 36. Macmillan, New York. 1934. $3.

[8] HRYNIEWIECKI, BOLESLAW, Précis de l'histoire de la botanique en Pologne. pp. 45. 57 portraits. Publ. by Soc. Bot. Pologne. Warsaw. (1931) 1934.

[9] GROUT, A. J., Moss flora of North America north of Mexico. Vol. III. Pt. 4. 4vo. pp. 179–277. pls. 45–80. Newfane, Vermont. Published by the author. 1934.

[10] BOT. GAZ. **88**:111. 1929.

[11] BOT. GAZ. **93**:110–111. 1932.

families, genera, species, and varieties covered by volume III is included with this part.

The aid of SEVILLE FLOWERS in illustrating numerous species is acknowledged by the author. None of the excellent line-drawn plates are duplications of illustrations in GROUT'S previous publication, Mosses with Hand-lens and Microscope.

Students of American mosses, as well as those who collect mosses as a hobby, will welcome the completion of this volume since it combines authenticity and convenience, and supplies what the older manuals, now out of print, cannot, a summary of our most recent knowledge of the mosses of the greater part of this continent.—P. D. VOTH.

Ferns of the northwest

In this volume,[12] both the lycopods and the ferns proper are included under the general title of ferns; and the northwest covers Washington, Oregon, Idaho, British Columbia, Montana, Wyoming, central and northern California. Taxonomic keys, a full bibliography, and a glossary make it easy for the layman to identify his local flora. The book is more than a taxonomic manual; for more than 30 years the author tramped the entire region covered by the work, and his field observations are recorded throughout. Even the taxonomic keys give the impression of the field rather than of the herbarium sheet. Many habitats are given, especially for Washington and in cases of rarer species. Throughout the book there are observations on variation which raise the question whether the differences are due to genetic relationship or to ecological conditions. The book will be useful to both the professional botanist and the layman who would know more about the ferns. It is copiously illustrated, by both drawings and photographs.—C. J. CHAMBERLAIN.

Illumination and growth

A recent study[13] has been made of the relation of various intensities of illumination to growth in the radish. The author grew plants in tents having one-ninth, one-third, one-half, three-fourths, and full daylight. She finds that the ratio of water to dry substance at most ages tends to decrease as the light is more intense; in other words, the percentage of dry matter is greater when plants are grown with strong illumination. Thus at one month under the intensities named the total dry weight percentages calculated from her figures are 4.8, 6.1, 7.5, 7.7, and 8.4. Not only are actual dry weight and dry weight percentage greater, but the mineral matter likewise increases with the illumination, increased light favoring absorption of mineral nutrients, as has already been

[12] FRYE, T. C., Ferns of the northwest. 8vo. pp. vi+178. Metropolitan Press, Portland, Oregon. 1934. $1.75.

[13] PANCHAUD, J., Action du milieu exterieur sur le métabolisme végetal. Rev. Gén. Bot. **46**: 586–603. 1934.

shown by the work of others. For growth in length and surface of body organs it is found that the lower illuminations are more favorable during early seedling stages while for older stages the higher light intensities serve best. With the faint illumination of one-ninth the radish plants spread their cotyledons early but produced no or few foliage leaves.—FRANCIS RAMALEY.

Wild flowers

Botanists and plant lovers in general will welcome a recent book.[14] It is essentially a reprinting in one volume of HOUSE's beautiful and well known work,[15] now practically unavailable. Aside from the addition of two new photographs in halftone and a rewritten introduction, the text and illustrations are the same in the two editions. The material has been compressed into one book through the use of lighter stock, and through the utilization of both sides of each page in the printing of the color plates. These 364 half-page and full-page color illustrations of the more striking herbaceous plants of northeastern United States have long been known as masterpieces of the photographers' and printers' arts. Issued under the auspices of a well known publishing house, this work should now have a wider circulation, and should do much to stimulate the layman's interest in our native plants.—C. E. OLMSTED.

Forest mensuration

Professional foresters and students will find this book,[16] the third to appear in the American Forestry series, a distinct contribution to forestry literature, owing to the fundamentally new method of approach. Modern methods in statistics and graphics are adapted and utilized to the fullest extent in showing how problems in measurement of timber crops may be solved. Numerous illustrative examples, as well as exercises and problems at the close of each chapter, enhance the value of the work as a textbook. Workers in other fields of biology in which biometrical methods are used will also find features of interest.—C. E. OLMSTED.

[14] HOUSE, HOMER D., Wild flowers. 4to. pp. 362. pls. 364. Macmillan, New York. 1934. $7.50.

[15] ———, Wild flowers of New York. Vols. I, II. Memoir 15, New York State Museum. pp. 362. Illus. Univ. of State of N.Y., Albany. 1918.

[16] BRUCE, DONALD, and SCHUMACHER, F. X., Forest mensuration. 8vo. pp. xiv+360. figs. 102. McGraw-Hill, New York. 1935. $3.50.